

I. References

A. Post-lethality Treatments and Antimicrobial Agents

Bedie, B. K., J. Samelis, J.N. Sofos, K. E. Belk, J. A. Scanga, and G. C. Smith . 2001. Antimicrobials in the formulation to control *Listeria monocytogenes* postprocessing contamination on frankfurters stored at 4° C in vacuum packages. J. Food Protect. 64:1949-1955

Butts, J. 2003. Seek & destroy: Identifying and controlling *Listeria monocytogenes* growth niches. Food Safety Mag. 9(2):24-9, 58.

Gande, N., and Muriana, P. M. 2002. Pre-package surface pasteurization of ready-to-eat meats with radiant heat oven for reduction of *Listeria monocytogenes*. Accepted for publication, Journal of Food Protection.

Glass, K. G., D. A. Granberg, A. L. Smith, A. M. McNamara, M. Hardin, J. Mattias, K. Ladwig, and E. A. Johnson. 2002. Inhibition of *Listeria monocytogenes* by sodium diacetate and sodium lactate on wieners and cooked bratwurst. J. Food Protect. 65: 116-123.

International Commission on Microbiological Specifications for Foods (ICMSF) 1996. Microorganisms in Foods 5 – Microbiological Characteristics of Food Pathogens, p. 148. (Blackie Academic & Professional, NY)

Janes, M. E., S. N Kooshesh and M.G. Johnson. 2002. Control of *Listeria monocytogenes* on the surface of refrigerated, ready-to-eat chicken coated with edible zein films containing nisin and calcium propionate. J. Food Sci. 67(No. 7): 2754-2757.

Muriana, P.M. and W. Quimby, C.A. Davidson, and J. Grooms. 2002. Postpackage pasteurization of ready-to-eat deli meats by submersion heating for reduction of *Listeria monocytogenes*. J. Food Protect.65:963-969.

Murphy, R.Y., L. K. Duncan, K.H. Driscoll, B.L. Beard, M. E. Berrang and J.A. Marcy. 2003. Determination of thermal lethality of *Listeria monocytogenes* in fully cooked chicken breast fillets and strips during post cook in-package pasteurization J. Food Protect 66:578-583.

Murphy, R.Y., L. K. Duncan, E. R. Johnson, M.D. Davis, R. E. Wolfe, and H. G. Brown. 2001. Thermal lethality of Salmonella senftenberg and Listeria innocua in fully cooked and packaged chicken breast strips via steam pasteurization. J. Food Protect. 64:2083-2087.

Murphy, R.Y., L. K. Duncan, K.H. Driscoll, and J.A. Marcy. 2003. Lethality of Salmonella and Listeria innocua in fully cooked chicken breast meat products during postcook in-package pasteurization. J. Food Protect. 66:242-248.

Murphy, R.Y., L.K. Duncan, K.H. Driscoll, J.A. Marcy, and B.L. Beard. 2003. Thermal inactivation of *Listeria monocytogenes* on ready-to-eat turkey breast meat products during post-cook in-package pasteurization via hot water. J. Food Protect. (accepted).

Porto, A.C.S., B. D. G. M. Franco, E.S. Sant'anna, J. E. Call, A. Piva, and J. B. Luchansky. 2002. Viability of a five-strain mixture of *Listeria monocytogenes* in vacuum-sealed packages of frankfurters, commercially prepared with and without 2.0 or 3.0% added potassium lactate, during extended storage at 4 and 10° C. J. Food Prot. 65:308-315.

PURAC America, Inc. Opti.Form Listeria Control Model. 2003. Personal Communication

Raghubeer, E.V. and E.D. Ting. 2003. The Effects of high hydrostatic pressure (HPP) on *Listeria monocytogenes* in RTE meat products. Avure Technologies, Inc. Submitted for publication.

Samelis, J. G.K. Bedie, J.N. Sofos, K.E. Belk, J.A. Scanga, and G.C. Smith. 2002. Control of *Listeria monocytogenes* with combined antimicrobials after postprocess contamination and extended storage of frankfurters at 4° C in vacuum packages. J. Food Protect. 65: 299-307.

Seman, D.L., A. C. Borger, J. D. Meyer, P. A. Hall, and A.L. Milkowski. 2002. Modeling the growth of *Listeria monocytogenes* in cured, ready-to-eat processed meat products by manipulation of sodium chloride, sodium diacetate, potassium lactate, and product moisture control. J. Food Protect 65:651-658.

Viskase Corporation. NOJAX® AL. 2003. Personal Communication.

B. Sanitation Guidelines

AMI. 1988. Interim guideline: microbial control during production of ready-to-eat meat products.

Anonymous. 2003. Sanitation systems and solutions. Food Safety 9(1):30-40, 45, 48-9.

Anonymous. 1999. Guidelines for developing good manufacturing practices (GMPs), standard operating procedures (SOPs), and environmental sampling/testing recommendations (ESTRs). Ready-to-Eat Products

Ednie, D. L., R. Wilson and M. Lang. 1998. Comparison of two sanitation monitoring methods in an animal research facility. *Comtem. Top. Lab. Anim. Sci.* 37(6):71-4.

Grau, F. H. 1996. Smallgoods and listeria . Food Australia. 48 (2): 81-83.

Huss, H. H., L. V. Jorgensen and B. F. Vogel. 2000. Control options for *Listeria monocytogenes* in seafoods. *Int. J. Food Microbiol.* 62:267-74.

International Commission on Microbiological Specifications for Foods (ICMSF). Microorganisms in Foods 7. Microbiological Testing in food Safety Management. 2002. Kluwer Academic/Plenum Publishers New York, New York.

Joint Task Force on Control of Microbial Pathogens. 1999. Interim guidelines: microbial control during production of ready-to-eat meat and poultry products.

Kohn, B. A., K. Costello and A. B. Philips. 1997. HACCP verification procedures made easier by quantitative Listeria testing. *Dairy Food Environ. Sanit.* 17(2):76-80.

Krysinski, E. P., L. J. Brown, and T. J. Marchisello. 1992. Effect of cleaners and sanitizers on *Listeria monocytogenes* attached to product contact surfaces. *J. Food Protect.* 55:(4):246-251.

Military standard: Sampling procedures and tables for inspection by attributes. 1963. MIL-STD-105E.

Moore, G. and C. Griffith. 2002. A comparison of surface sampling methods for detecting coliforms on food contact surfaces. *Food Microbiol.* 19:65-73.

National Advisory Committee on the Microbiological Criteria for Foods. 1991. *Int. J. Food Microbiol.* 14(3/4):232-37.

Seeger, K. and M. W. Griffiths. 1994. Adenosine triphosphate bioluminescence for hygiene monitoring in health care institutions. *J. Food Prot.* 57(6);509-12.

Silliker Laboratories. 1996. Smart sanitation: principles and practices for effectively cleaning your plant. Video.

Suslow, T. and L. Harris. Guidelines for Controlling *Listeria monocytogenes* in Small- to Medium-Scale Packing and Fresh Cut Operations. 2000. University of California Publication 8015.

Tompkin, R. B., V. N. Scott, D. T. Bernard, W. H. Sveum, and K. S. Gombas. 1999. Guidelines to Prevent Post Processing Contamination from *Listeria monocytogenes*. *Dairy, Food and Environmental Sanitation.* 19 (8): 551-562.

Tompkin, R. B. 2002. Control of *Listeria monocytogenes* in the Food-Processing Environment. J. Food Prot. 65(4):709-25.

ATTACHMENT 1

CONTROL REQUIREMENTS for *LISTERIA MONOCYTOGENES*

Requirements	→ Increasing Risk Levels and Verification Testing →				
	ALTERNATIVE 1	ALTERNATIVE 2		ALTERNATIVE 3	
	Post-lethality Treatment AND Antimicrobial agent or Process	Post-lethality Treatment OR Antimicrobial agent or Process	Post-lethality Treatment	Antimicrobial Agent or Process	Sanitation and Testing Program
				Non-deli, Non-hotdog	Deli or hot-dog product
Validate effectiveness of post-lethality treatment	X	X			
Document effectiveness of antimicrobial agent or process	X		X		
Sanitation Program Requirements			X	X	X
Testing food contact surfaces (FCS)			X	X	X
State testing frequency			X	X	X
Identify size and location of sites to be sampled			X	X	X
Explain why testing frequency is sufficient			X	X	X
Identify conditions for Hold-and-Test, when FCS (+)			X	X	X
Additional Sanitation Program Requirements					X
Follow-up testing to verify corrective actions are effective after 1 st FCS (+)					X
If follow-up testing yields 2 nd FCS (+), hold products that may be contaminated until problem is corrected as shown by FCS (-) in follow-up testing.					X
Hold and test product lots for <i>L. monocytogenes</i> using sampling plan that provides statistical confidence. Release, rework or condemn products based on results. Document results and product disposition.					X

OTHER REQUIREMENTS

- Post-lethality treatments must be included in the HACCP plan.
- Antimicrobial agents must be included either in the HACCP plan, Sanitation SOP, or prerequisite program.

- Sanitation programs must be included either in HACCP plan, Sanitation SOP, or prerequisite program. If in the Sanitation SOPs or prerequisite program, there must be supporting documentation for the hazard analysis determination that this hazard is not reasonably likely to occur.
- Verification testing for sanitation in the post-lethality environment may be for *Listeria monocytogenes*, *Listeria* spp. or *Listeria*-like organisms.
- Product testing must be confirmed for *Listeria monocytogenes*.

- Establishment must maintain sanitation in the post-lethality environment per 9 CFR 416.
- If *L. monocytogenes* controls are in HACCP plan, establishment must validate and verify effectiveness per 9 CFR 417.4
- If *L. monocytogenes* controls are in Sanitation SOPs, their effectiveness must be evaluated per 9 CFR 416.14.
- If *L. monocytogenes* controls are in prerequisite programs, the program and results must be included in documentation required by 9 CFR 417.5
- Establishment must make verification results available to inspection program personnel.

ATTACHMENT 2

CHART OF RTE VS NRTE PRODUCTS

TYPE	CLASS	PROCESSING CATEGORY	ISP CODE	REG REQUIRED SAFETY LABELING	WHAT THE HAZARD ANALYSIS/HACCP PLAN MAY ADDRESS
A product containing a meat/poultry product (in whole or in part) which has not received an adequate lethality treatment for pathogens (i.e. raw or partially cooked product).	Not-ready-to-eat	<ul style="list-style-type: none"> • Raw Product Ground – ISP 03B • Raw Product Not Ground – ISP 03C • Not Heat Treated Shelf Stable – ISP 03E • Heat Treated –shelf stable – ISP 03F • Heat Treated but not Fully Cooked Not Shelf Stable - ISP 03H • Products with secondary inhibitors Not Shelf Stable – ISP 03I • 		Product must be labeled with statements such as keep refrigerated, keep frozen, or refrigerate leftovers. Use of Safe Handling Instruction (SHI) labeling required.	<ul style="list-style-type: none"> • Use of SHI labeling (Some establishments may have a CCP for SHI labeling application). <p>If it is not obvious that the product is raw and needs to be cooked:</p> <ul style="list-style-type: none"> • Features on labeling are conspicuous so that intended user is fully aware that product must be cooked for safety. This is best conveyed through the product name (e.g., “Cook and Serve”) but may also be conveyed by the use of an asterisk on the product name that is associated with a statement on the principle display panel, or by a burst stating such things as “needs to be fully cooked,” “see cooking instructions,” or “cook before eating.” • Validation that: <ol style="list-style-type: none"> a. Cooking and preparation instructions on the product are sufficient to destroy pathogens. b. Instructions are realistic for the intended consumer.
A product containing a meat/poultry component that has received a lethality treatment for pathogens in combination with non-meat/poultry components that need to receive a lethality treatment by the intended user. This includes meals, dinners, and frozen entrees.	Not-ready-to-eat	<ul style="list-style-type: none"> • Heat Treated but not Fully Cooked Not Shelf Stable - ISP 03H 		Product must be labeled with statements such as keep refrigerated or frozen. Use of SHI labeling is recommended.	<ul style="list-style-type: none"> • Validation that: <ol style="list-style-type: none"> a. The meat/poultry component received an adequate lethality treatment for pathogens. b. Cooking and preparation instructions on the product are sufficient to destroy pathogens. c. Instructions are realistic for the intended consumer. • Features on labeling are conspicuous so that intended user is fully aware that product must be cooked for safety. This is best conveyed through the product name (e.g., “Cook and Serve”) but may also be conveyed by the use of an asterisk on the product name that is associated with a statement on the principle display panel, or by a burst stating such things as “needs to be fully cooked,” “see cooking instructions,” or “cook before eating.” • If necessary, hazard analysis should address whether instructions on the label are needed related to cross-contamination (e.g., avoid contact of contents) and prevention of pathogenic growth (e.g., promptly refrigerate leftovers). <p>NOTE: Inspection program personnel are to collect samples as RTE if the establishment does not follow the guidance above.</p>

<p>A product containing a meat/poultry component that has received a lethality treatment for pathogens that may or may not be in combination with a non-meat/ poultry component that does not need to receive a lethality treatment by the intended user.</p>	<p>Ready-to-eat</p>	<ul style="list-style-type: none"> • Not Heat Treated Shelf Stable – ISP 03E • Heat Treated Shelf Stable – ISP 03F • Fully Cooked Not Shelf Stable – ISP 03G • Products with secondary inhibitors Not Shelf Stable – ISP 03I 	<p>If the product is not shelf stable labeling such as keep refrigerated or frozen is required.</p>	<ul style="list-style-type: none"> • See part 417 of the meat and poultry regulations.
---	---------------------	--	---	---

ATTACHMENT 3

PRODUCTION INFORMATION ON POST-LETHALITY EXPOSED READY-TO-EAT PRODUCTS
SAMPLE FORM (DRAFT)

SEE PAGE 2 FOR INSTRUCTIONS ON COMPLETING AND SUBMITTING THE FORM. (Press the "Page Down" button to go to Page 2)

According to the Paperwork Reduction Act of 1995, an agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a valid OMB control number. The valid OMB control number for this information collection is 0583-00_-. The time required to complete this information collection is estimated to average 60 minutes per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information.

U.S. DEPARTMENT OF AGRICULTURE FOOD SAFETY AND INSPECTION SERVICE			1 <u>Deli product</u> : A ready-to-eat meat or poultry product that typically is sliced, either in an official establishment or after distribution from an official est., & typically is assembled in a sandwich for consumption (9 CFR 430.1). Examples include: ham, bologna, chicken roll, turkey breast, olive loaf				4 Examples include dry salami, Lebanon bologna, cervelat, thuringer, summer sausage, pepperoni						
PRODUCTION INFORMATION ON POST-LETHALITY EXPOSED READY-TO-EAT PRODUCTS			2 <u>Hot dog product</u> : A ready-to-eat meat or poultry frank, frankfurter, or weiner such as a product defined in 9 CFR 319.180 and 319.181 (9 CFR 430.1). Examples include hot dogs, wieners, frankfurters		5 Examples include jerky, dried beef, dried duck breast, basturma, carna seca		6 Examples include countrycured ham, prosciutto, dry cured duck, coppa, cappicola						
See Instructions on Page 2.			2. EST. NO.		3 Examples include chicken nuggets, chili, fully cooked bacon, frozen dinners/entrees								
1. ESTABLISHMENT NAME			3a. STREET ADDRESS (P.O. Box alone not acceptable)		DELI PRODUCTS ¹		OTHER THAN DELI OR HOTDOG PRODUCTS						
3b. CITY			3c. STATE		3d. ZIP CODE		SLICED AND PACKAGED AT OFFICIAL EST.	TO BE SLICED AFTER DISTRIBUTION FROM OFFICIAL EST.	HOT DOG PRODUCTS ²	FULLY COOKED PRODUCTS ³	FERMENTED PRODUCTS (with or without cooking) ⁴	DRIED PRODUCTS ⁵	SALT-CURED PRODUCTS ⁶
POST-LETHALITY EXPOSED PRODUCT ALTERNATIVES													
ALTERNATIVE 1													
ANNUAL PRODUCTION VOLUME (lbs.) ➔													
BOTH A POST-LETHALITY TREATMENT AND AN ANTIMICROBIAL AGENT OR PROCESS USED.													
Least log reduction of L_m achieved by the post-lethality treatment: a. 2 log or greater b. < 2 log to 1 log c. < 1 log d. don't know													
Highest increase in growth of L_m allowed by the antimicrobial agent or process a. 1 log or less b. > 1 log to 2 log c. > 2 log d. don't know													
Lowest frequency of verification by testing food contact surface per line per year: a. > 2 times b. 2 times c. less than 2 times d. don't know													
ALTERNATIVE 2													
ANNUAL PRODUCTION VOLUME (lbs.) ➔													
ONLY A POST-LETHALITY TREATMENT													
Least log reduction of L_m achieved by the post-lethality treatment: a. 2 log or greater b. < 2 log to 1 log c. < 1 log d. don't know													
Lowest frequency of verification by testing food contact surface per line per year: a. > 4 times b. 4 times c. less than 4 times d. don't know													
OR													
ONLY AN ANTIMICROBIAL AGENT OR PROCESS USED.													
Highest increase in growth of L_m allowed by the antimicrobial agent or process a. 1 log or less b. > 1 log to 2 log c. > 2 log d. don't know													
Lowest frequency of verification by testing food contact surface per line per year: a. > 4 times b. 4 times c. less than 4 times d. don't know													
ALTERNATIVE 3													
ANNUAL PRODUCTION VOLUME (lbs.) ➔													
ONLY SANITATION PROGRAM USED.													
Lowest overall effectiveness of routine cleaning and sanitation: a. > 87.5 % b. 87.5 % c. < 87.5 % d. don't know													
Lowest frequency of verification by testing food contact surfaces per line per month: (Very small HACCP size category establishments) a. > 1 time b. 1 time c. < 1 time d. don't know													
Small HACCP size category establishments: a. > 2 times b. 2 times c. < 2 times d. don't know													
Large HACCP size category establishments: a. > 4 times b. 4 times c. < 4 times d. don't know													
4. PRINT NAME/TITLE OF AUTHORIZED ESTABLISHMENT OFFICIAL							5. SIGNATURE OF AUTHORIZED ESTABLISHMENT OFFICIAL					DATE	

DRAFT

DRAFT

ESTIMATES OF ANNUAL PRODUCTION VOLUME

- FSIS collects estimates of the annual production volume and related information on post-lethality exposed ready-to-eat (RTE) meat and poultry products. Establishments that produce these products are required by 9 CFR 430.4(d) to make this information available to FSIS at least annually. FSIS uses the information as a basis for directing its verification activities, including microbiological sampling, at affected establishments.

- The regulations classify the products by the Listeria control alternative used:

ALTERNATIVE 1: establishment uses a post-lethality treatment and an antimicrobial agent/process

ALTERNATIVE 2: establishment uses either a post-lethality treatment or an antimicrobial agent/process

ALTERNATIVE 3: establishment uses sanitation and testing program and use neither a post-lethality treatment nor an antimicrobial agent/process

Note: An antimicrobial agent/process can be considered a post lethality treatment if it reduces the level of *L. monocytogenes* in the post-lethality exposed product (e.g. growth inhibitor packaging). The establishment must validate, document and verify the reduction.

Examples of post-lethality treatments are steam pasteurization, hot water pasteurization, high pressure process.

Examples of antimicrobial agents are sodium diacetate, potassium lactate, and growth inhibitor packaging

Examples of antimicrobial processes are freezing or drying

DRAFT

INSTRUCTIONS FOR COMPLETING THE FORM:

ITEMS 1 - 3d

- Enter establishment's name, number and address.

ALTERNATIVE 1 - ALTERNATIVE 3

- Enter your establishment's annual production volume (lbs.) of post-lethality exposed RTE meat and poultry products for each Alternative in each applicable product category column. The notes above the product category columns give examples of products for the product category in each column.
- In each product category column, where applicable, enter the letter that most nearly answers the question about your establishment's control of *L. monocytogenes* (*Lm*), the log reduction or growth limitation achieved, and the frequency of food-contact surface verification testing. Please refer to your HACCP plan, Sanitation SOP or other prerequisite program to verify the control method used.
- If your establishment uses Alternative 3, enter in the appropriate product category column, the letter(s) corresponding to the effectiveness of cleaning and sanitation and to the frequency of food-contact surface testing.

ITEMS 4 - 5

- Print Name and Title of Authorized Official
- Signature and Date of Authorized Official

SUBMIT THE COMPLETED FORM TO THE FOLLOWING ADDRESS: FSIS-USDA-Data Analysis and Statistical Support Staff
201 Cotton Annex
300 12th Street, SW
Washington, DC 20250

- Please send a revised form anytime there is a significant change in the Alternative category or volume of production.

ATTACHMENT 4
STUDIES ON POST-LETHALITY TREATMENTS
And ANTIMICROBIAL AGENTS

A. Studies on Post-lethality Treatments

(Mention of trade marks or commercial names does not constitute endorsement by USDA)

I. Steam Pasteurization and Hot Water Pasteurization

Post processing contamination of RTE meat and poultry is mostly confined to the surface. Pasteurization by steam and hot water acts on the surface microbial contaminants by the action of heat. Studies on surface pasteurization using steam or hot water were shown to be effective in reducing this contamination.

Studies by Murphy et al. (2003a) showed that post-cook hot water pasteurization and steam pasteurization resulted in a 7 log₁₀ reduction of *L. monocytogenes* in inoculated vacuum packaged fully cooked sliced chicken. The reduction was effective when single – packaged breast fillets, 227 g- package strips and 454 g-packaged strips were heat treated at 90 C in a continuous steam cooker or hot water cooker for 5, 25 and 35 minutes respectively. These investigators developed a model called ThermoPro that could predict the thermal lethality of pathogens in fully cooked meat and poultry products during post-cook in-package pasteurization (Murphy et al., 2001, 2003b, 2003c). The model was developed using *L. innocua* and verified for *L. monocytogenes*.

II. Pre-Package Pasteurization and Post-Package Surface Pasteurization

Pre-package surface pasteurization treatment of fully cooked meat removed from their packaging wrap and inoculated with *L. monocytogenes* resulted in a 1.25 to 3.5 log reduction with a treatment time of 60-120 sec at 475 to 750° F air temperature (Gande and Muriana, 2003). Surface pasteurization was applied on cooked whole and split roast beef, whole corned beef, and whole and formed ham using a radiant oven (“Infrared Grill”, Unitherm FoodSystems). Pre-package pasteurization (60 sec) combined with post-package submerged water pasteurization using formed ham (60 or 90 sec), turkey bologna (45 or 60 sec), and roast beef (60 or 90 sec), resulted in a 3.2 to 3.9 log reduction for ham, 2.7-4.3 log reduction for bologna, or a 2.0-3.75 log reduction for roast beef. The level of reduction varied depending on the method of inoculation, type of product used, treatment temperature, and residence time.

Muriana et al., (2002) used a stainless steel water bath (similar to the Unitherm commercial Aquaflo food processor) to submerge cooked RTE deli-style whole or formed turkey, ham and roast beef, removed from their package, inoculated with *L.*

monocytogenes and vacuum packaged. Results show a 2-4 log decrease in the levels of *L. monocytogenes* in inoculated products post-cooked at 195-205° F for 2-10 min.

III. High Hydrostatic Pressure Processing

High pressure processing (HPP) is one of the new technologies used for food processing. This technology provides a means of ensuring food safety for those products that are difficult to be heat treated due to organoleptic effects. HPP was shown to inactivate pathogens without any thermal or chemical effects and at the same time preserve the quality of the product. Raghubeer and Ting (2003) evaluated the efficacy of high hydrostatic pressure processing in inactivating *L. monocytogenes* in retail-packaged samples of sliced ham, turkey and roast beef obtained from a manufacturer and repackaged in 25-g portions. Results show that an inoculum of about 10^4 *L. monocytogenes* cocktail in these 3 products and HPP treatment at 87,000 psi for 3 minutes showed no recovery of *L. monocytogenes* after 61 days of storage at 34° F. There were no pressure-injured cells detected. There were no adverse organoleptic effects detected on the 3 HPP treated products during the 61-day shelf life study. No signs of spoilage were seen on all 3 products after 61 days of storage, and for 100 days for ham and turkey. According to the investigators, the normal shelf life of these products is 30 days, so the HPP treatment extended the shelf life of the products.

B. Studies on the Use of Antimicrobial Agents

I. Addition of Lactates, Acetates, Diacetates to Meat Formulations

Studies have shown that lactic acid and acetic acid have significant antimicrobial activity in broth and food systems. Sodium and potassium salts of these acids, when added to processed meat formulations are also known to potentially inhibit pathogenic bacteria especially *L. monocytogenes*. These antimicrobials inhibit growth of pathogens by inhibiting their metabolic activities. Interest in these antimicrobials is in the growth inhibition of *L. monocytogenes* in post lethality exposed RTE meat and poultry products.

FSIS recently increased the permissible levels of sodium acetate as a flavor enhancer in meat and poultry products, and of sodium diacetate as a flavor enhancer and as an inhibitor of pathogen growth to 0.25 % (65 FR 3121-3123/2000). The rule also permitted the use of sodium lactate and potassium lactate in fully cooked meat, meat food products, poultry, and poultry food products, except for infant foods and formulas at levels of up to 4.8 % of total product formulation for the purpose of inhibiting the growth of certain pathogens. Approved antimicrobials for meat and poultry products can be found in 9 CFR 424.21. The addition of antimicrobials in the formulation must be included in the ingredient statement of the label. Several studies used these antimicrobials to show their ability to inhibit growth of *L. monocytogenes* in different meat formulations.

Seman et al., (2002) developed a mathematical model capable of predicting the growth or stasis of *L. monocytogenes* in commercial cured meat products using a response surface method. The model can be used by manufacturers in the determination of the appropriate

amounts of potassium lactate and sodium diacetate to be added to cured meat products that are organoleptically sensible and will not support the growth of *L. monocytogenes*. Thirty products were formulated by using a variety of raw material sources such as pork trimmings, trimmed turkey breast halves and four-muscle ham. Varying amounts of potassium lactate and sodium diacetate were added to the meat formulation and the meats were processed into different products. After chilling, the products were stripped of their casings, sliced into 25-g slices, placed into pouches, and inoculated with *L. monocytogenes* by applying to the surface of 100g of cured meat (four slices).

The results show that increasing amounts of potassium lactate syrup and sodium diacetate decreased the growth rate of *L. monocytogenes*, while increasing finished product moisture increased the growth rate. Sodium chloride content was not significant but was found to have a negative correlation to growth rate. The investigators provided a final regression equation predicting the growth of *L. monocytogenes* in cured RTE meat products stored at 4° C. The investigators used predictive model performance factors and a simple linear regression analysis to evaluate the model generated in this study. They verified the accuracy of the model by comparing with actual *L. monocytogenes* growth data from an independent challenge study conducted with four different commercial RTE meat products using similar storage conditions. Performance factors calculated and evaluated for control products (those not containing potassium lactate and sodium diacetate) indicated that on the average, the predicted growth of *L. monocytogenes* exceeded those of the observed values by about 24 %.

This study provided a useful model in determining the target amounts of potassium lactate and sodium acetate for cured meat product formulations to inhibit the growth of *L. monocytogenes*. The calculations would also require knowledge of the finished product sodium chloride and moisture contents. The investigators advised that this validated model is specific to the products designed for the study and the *L. monocytogenes* strains used. Testing of this model in other environments and with other *Listeria* spp., and to formulations that are outside the model's limits may result in different maximum growth rates. This study was used as the basis for the Opti.Form *Listeria* Control Model.

The Opti.Form Listeria Control Model (PURAC) is a unique tool to calculate the levels of lactate and diacetate required to retard the growth of *Listeria monocytogenes* in cured meat and poultry products. The model is based on the study detailed in the paper by Seman et al, 2002, above. The model, which is available on CD-Rom includes:

- instructions on how to use the model
- explanation on the development of the model
- information on the anti-microbial effect of lactate and diacetate
- lactates and diacetates and use of these products
- regulations and labeling
- literature references

To receive a free copy of the model on CD-Rom, call: 888-899 8229, E-mail pam@purac.com

Bedie et al., (2001) evaluated the use of antimicrobials, included in frankfurter formulations, on *L. monocytogenes* populations during refrigerated storage. Fully cooked and cooled frankfurters were inoculated with 10^3 to 10^4 CFU /cm² of *L. monocytogenes* after peeling and before vacuum packaging. Samples were stored at 4° C for up to 120 days and sampled for testing on assigned days. Results are as follows:

ANTIMICROBIAL	LEVEL (%)	<i>L. MONOCYTOGENES</i> GROWTH INHIBITION
Sodium lactate	3	70 days no pathogen growth
Sodium diacetate	0.25	50 days no pathogen growth
Sodium acetate	0.25, 0.50	20 days no pathogen growth
Sodium lactate	6	120 days no growth and reduced pathogen growth
Sodium diacetate	0.5	120 days no growth and reduced pathogen growth
Inoc. Control	0.0	Increased to 6 logs in 20 days

Note: Sodium acetate is approved as a flavor enhancer, not as an antimicrobial agent.

No pathogen growth refers to no increase in the number of inoculated *L.monocytogenes* cells (bacteriostatic); while reduced pathogen growth refers to a decrease in the number of inoculated *L. monocytogenes*_ cells (bactericidal) in the product. In this study, tables showed the reduction varied with storage days, but was up to 1.0 log on some days. Antimicrobials were found to have no effect on pH except for sodium diacetate at 0.5 % which reduced the initial pH. Using the formulations and conditions in the study, establishments can add 3 % sodium lactate in the frankfurter formulation and obtain no growth of *L. monocytogenes* up to 70 days at refrigerated storage of 4° C. If the lethality treatment is adequate to eliminate *L. monocytogenes*, then the only probable source of *L. monocytogenes* would be from exposure of the product during peeling and repackaging. However, the establishment's sanitation program may keep the numbers to a very low level, and 3 % sodium lactate included in the formulation would inhibit the growth of *L. monocytogenes* during the product's refrigerated shelf life. Levels of sodium lactate at 6.0 % and sodium diacetate at 0.5 % showed a reduction of the pathogens, however these levels are above the permitted levels.

This study by Samelis et al., (2002) used similar treatments, processing and inoculation procedures and frankfurter formulations as the previous study described above. However, in this study combinations of antimicrobials were used, and in combination with hot water treatment. Hot water treatment involved immersion of frankfurters, with two product links in a package to 75 or 80° C for 60 s. Storage at 4° C shows:

<u>TREATMENT</u>	<u>LEVELS (%)</u>	<u><i>L. MONOCYTOGENES</i> GROWTH INHIBITION</u>
Sodium lactate	1.8	35-50 days no growth
Sodium lactate + sodium acetate	1.8 0.25	120 days no growth; 35-50 days growth reduction
Sodium lactate + Sodium diacetate	1.8 0.25	120 days no growth; 35-50 days growth reduction
Sodium lactate +	1.8	120 days no growth, 35-50 days growth

Glucuno-delta-lactone	0.25	reduction
Hot water treatment (80° C, 60 s) + Sodium lactate	1.8	Inoc. population reduced by 0.4-0.9 log CFU/cm ² , and 50-70 days growth reduction by 1.1-1.4 CFU/cm ²
Hot water treatment (80° C, 60 s)		Increase in growth to about 6-8 logs in 50 days
Inoculated Control, no treatment		Increase in growth to about 6 logs in 20 days and 8 logs thereafter up to 120 days

Note: Sodium lactate was used as a 3 % of a 60 % (wt/wt) commercial solution.

Glucuno-delta lactone is approved as an acidifier, and a curing accelerator, but not as antimicrobial. Sodium acetate is approved as a flavor enhancer, not as an antimicrobial agent.

Glass et al., (2002) evaluated sodium lactate and sodium diacetate on wieners and cooked bratwurst containing both beef and pork supplied by a commercial manufacturer.

Antimicrobial solutions used were sodium lactate and sodium diacetate singly or in combination at varying concentration. Wieners were repackaged in gas-impermeable pouches, then surface-inoculated with *L. monocytogenes* mixture on multiple areas of the surface of each link. Packages were vacuum-sealed and stored at 4.5° C for up to 60 days. Two types of cooked bratwurst from a commercial manufacturer were evaluated: bratwurst that was cured and naturally smoked and bratwurst that was uncured and unsmoked. Bratwurst was stored at 3 or 7° C for up to 84 days.

The surface treatment consisting of dipping wieners into solutions containing up to 6 % lactate and up to 3 % diacetate for 5 s did not delay pathogen growth, indicating that dipping wieners in the lactate/diacetate solutions is not an efficient way to apply the antimicrobials. However, the inclusion of lactates and diacetates in the formulation was found effective in inhibiting growth of *L. monocytogenes*. Results are as follows:

<u>PRODUCT</u>	<u>Sodium Lactate (%)</u>	<u>Sodium diacetate (%)</u>	<u><i>L. monocytogenes</i> levels (CFU/pkg)</u>
Bratwurst uncured, unsmoked	3.4	0.1	Growth delayed for 4-12 weeks at 7 and 3° C storage, respectively.
	2.0	0.0	Growth delayed for 1-2 weeks at 7 and 3° C
Bratwurst cured, smoked	3.4	0.1	Growth inhibited for 12 weeks at 7 and 3° C
	0.0	0.0	Growth up to 1 log after 4 weeks at 7 and 3° C
Wieners	3.0	0.0	Growth inhibited for 60 days at 4.5° C
	1.0	0.1	Growth inhibited for 60 days at 4.5° C

Study by (Porto et al., 2002) used freshly processed peeled frankfurters in vacuum sealed packages obtained from a commercial manufacturer. Two formulations of links were used in the study: one with added 2 or 3 % potassium lactate and the other without added potassium lactate. Frankfurters were aseptically removed from their original package, repackaged, and inoculated with a mixture of *L. monocytogenes*. The packages were vacuum-sealed to 95 kPa and incubated at 4 and 10° C.

Results show that addition of 2 % or 3 % potassium lactate in frankfurters can appreciably enhance safety by inhibiting or delaying the growth of *L. monocytogenes* during storage at refrigeration or abused temperatures. The viability of the pathogen was influenced by pH, and the levels of lactate added, but not by the presence of indigenous lactic acid bacteria.

Potassium lactate (%)	Inoculum CFU/pkg	Storage temp °C	Days Storage	<i>L. monocytogenes</i> levels (CFU/package)
2.0	20	4	90	Remained at about 1.6 log
3.0	20	4	90	Remained at about 1.4 log
3.0	500	4	90	Remained at about 2.4 log
0.0	20	4	90	Increased to about 4.6 log
0.0	500	4	90	Increased to about 5.0 log
2.0	20	10	60	Remained at about 1.4 log
3.0	20	10	60	Remained at about 1.1 log
0.0	20	10	60	Increased to about 6.5 after 28 days, declined to about 5.0 after 60 days
3.0	500	10	60	Remained at about 2.4
0.0	500	20	60	Increased to about 6.6 log after 40 days and declined to about 5.5 log after 60 days

II. Growth Inhibitor Packaging

Growth inhibitor packaging is an intervention, which delivers an active antibacterial agent to the surface of an encased sausage product. By incorporating this special coating onto the internal surface of cellulose casings, the antilisterial treatment is transferred to the surface of the processed meat/sausage during thermal processing. Upon removal of the casing, the treatment remains active on the meat surface, providing effective protection against inadvertent *Listeria* contamination during subsequent peeling and packaging processes. Growth inhibitor packaging used in conjunction with functional HACCP and Good Manufacturing Practices provides the industry with one more tool in their intervention strategy to control the risk of pathogen contamination in ready-to-eat meat and poultry products.

Studies on meat formulations for hot dogs using NOJAX[®] AL[™] (Viskase) showed that use of the casings provide a lethality hurdle to the growth of *Listeria monocytogenes*, not just an inhibitory effect. The lethality impact is delivered within the first hours/days of the sausage/hot dog package life. This impact is dependent on many variables but is generally in the range of 1 – 2 log kill of *L. monocytogenes* at high levels of inoculation.

This performance has been observed in challenge studies conducted on hot dogs drawn from commercial full-scale trials at a number of commercial processing plants. In high inoculation trials, NOJAX AL has been combined with conventional growth inhibiting additives, and as expected, the lethality impact is obtained and then maintained throughout the product life cycle. In these same trials, without growth inhibiting additives, this casing produces lethality but in several weeks the remaining *L. monocytogenes* begin to grow.

NOJAX AL is available in the U.S. having approval by both FDA and USDA for its key component, nisin. This GRAS component must be included in the ingredient statement via a label change request to the FSIS Labeling and Consumer Protection Staff. Because this is a naturally derived polypeptide, there are storage and use-by criteria that will have to be adhered to by the user for maximum benefit. Casing shelf-life is about 60-90days with a not to exceed 85° F.

This technology can be applied to most hot dogs and sausages that are encased in cellulose casing. This casing intervention can be used in any instance where casing is used as a mold for processed meat and poultry during thermal processing. This would include cellulose, plastic, and possibly natural casing. As part of a manufacturer's decision to use this technology, benefits are: 1) no capital costs or new equipment; 2) no change in processing steps, plant reconfigurations or introduction of process bottlenecks—essentially processor transparent in all aspects of use except casing storage requirements; 3) no impact on flavor, texture, or package appearance, and 4) minor labeling change to ingredient statement

Since this is a surface treatment, cost will be proportional to the surface to volume ratio of the product: the larger the sausage diameter, the lower the cost per pound. In general, economic analyses put the cost of this lethality intervention at about 2-3 cents per pound of finished product, with a mid-range target price of 2.5 cents per pound for a traditional 10-to-the-pound retail pack of hot dogs.

Janes et al., (2002) investigated the effect of nisin added to zein film coatings (Z) coated onto cooked ready-to-eat chicken against *L. monocytogenes*. Cooked chicken samples inoculated with *L. monocytogenes* were dipped into Z dissolved in propylene glycol or ethanol, with or without added nisin (1,000 IU/g) and/or 1 % calcium propionate and stored at 4 C or 8 C for 24 days. After 16 d at 4 C, *L. monocytogenes* was suppressed by 4.5 to 5 log CFU/g with zein film coatings with nisin. The most effective treatment in the study for controlling *L. monocytogenes* on the surface of ready-to-eat chicken was using edible zein film coatings containing nisin at a storage temperature of 4°C.

The use of film coatings in a processing plant would be to fully process the meat products then coat them with the films. Coating can be done by spraying or dipping the processed meat products and then allowing them to dry. Zein coatings on the meat products can be dried by circulating air around the meat product using a fan. Finally, the dried coated meat products can be packaged with the usual plastic film material and refrigerated. This study has not been tested in commercial poultry processing conditions.

Some general observations from the published studies on antimicrobials:

- Lactates, acetates and diacetates were found more effective in inhibiting growth of *L. monocytogenes* when used in combination than when used singly.
- These antimicrobials were found more effective when used to the maximum allowable concentration. However, higher concentrations of antimicrobials used in the formulation may affect the sensory qualities of the product, such as flavor and texture, which would necessitate sensory evaluation of treated products.
- When used in combination, the amount needed to inhibit growth may be reduced.
- These antimicrobials were found to have listeristatic activity more than listericidal activity, i.e. they prevent growth of the pathogen more than reduce the number of cells of the pathogen, and therefore may not be effective against gross contamination of a product. The establishment's sanitation program should control gross contamination of the processing environment and equipment. Addition of antimicrobials would be effective only as part of the overall HACCP strategy.
- Including these antimicrobials in the formulation was found to be more effective in inhibiting listerial growth than dipping products in solutions of antimicrobials.
- The antimicrobial activity of lactates and diacetates when used singly or in combination is affected by the level of contamination of the meat product surface, and processing factors such as pH, moisture, water activity, fat, nitrite, salt content, time and temperature of storage, and packaging atmosphere.
- Application of the treatments used in these studies is limited to the formulations, products and treatments used in the studies. Applying these studies to other products and formulations may result in different rates of growth inhibition. Therefore the effectiveness of the antimicrobials used in these studies must be verified by the establishment for other processed meat products and other storage temperatures.
- Antimicrobials used in the formulation must have an effective antilisterial activity throughout the commercial shelf life of the product. Currently the targeted commercial shelf life of refrigerated cooked meat products in the U.S.A. is 75 to 90 days.
- Using post-packaging thermal treatments in addition to antimicrobials was found to increase the total antilisterial effects of the antimicrobials.
- These antimicrobials were found to be more effective in smoked products formulated with sodium nitrite, or in products stored at strict refrigeration temperatures.
- Use of these antimicrobials may be a cost effective antilisterial method that very small establishments can use.

ATTACHMENT 5

USING the ICMSF (International Commission on Microbiological Specifications for Foods) SAMPLING PLAN

ICMSF classifies 15 different cases of sampling plans, with sampling plan stringency based on degree of risk and the effect on risk of the conditions of use. Case 13, 14, or 15 would apply to the severe category of microbial hazards, including *L. monocytogenes*. In case 13, where conditions of use reduce risk (e.g., food will be fully cooked), the sampling plan is $n=15, c=0$. (n is the number of samples; $c=0$ means that none of the “ n ” samples can be positive for the test organism, in this case *L. monocytogenes*.) For case 14, conditions cause no change in the hazard (e.g., frozen storage), and $n=30, c=0$. For case 15, conditions may increase the risk (e.g., foods subjected to conditions that allow growth; $n=60$ and $c=0$). Note that product samples can be composited.

The following are examples of statistically derived sampling plans that can be used for sampling products under hold-and-test. The number of samples would be as specified for these cases based on the risk of the product. Examples for the categories are included.

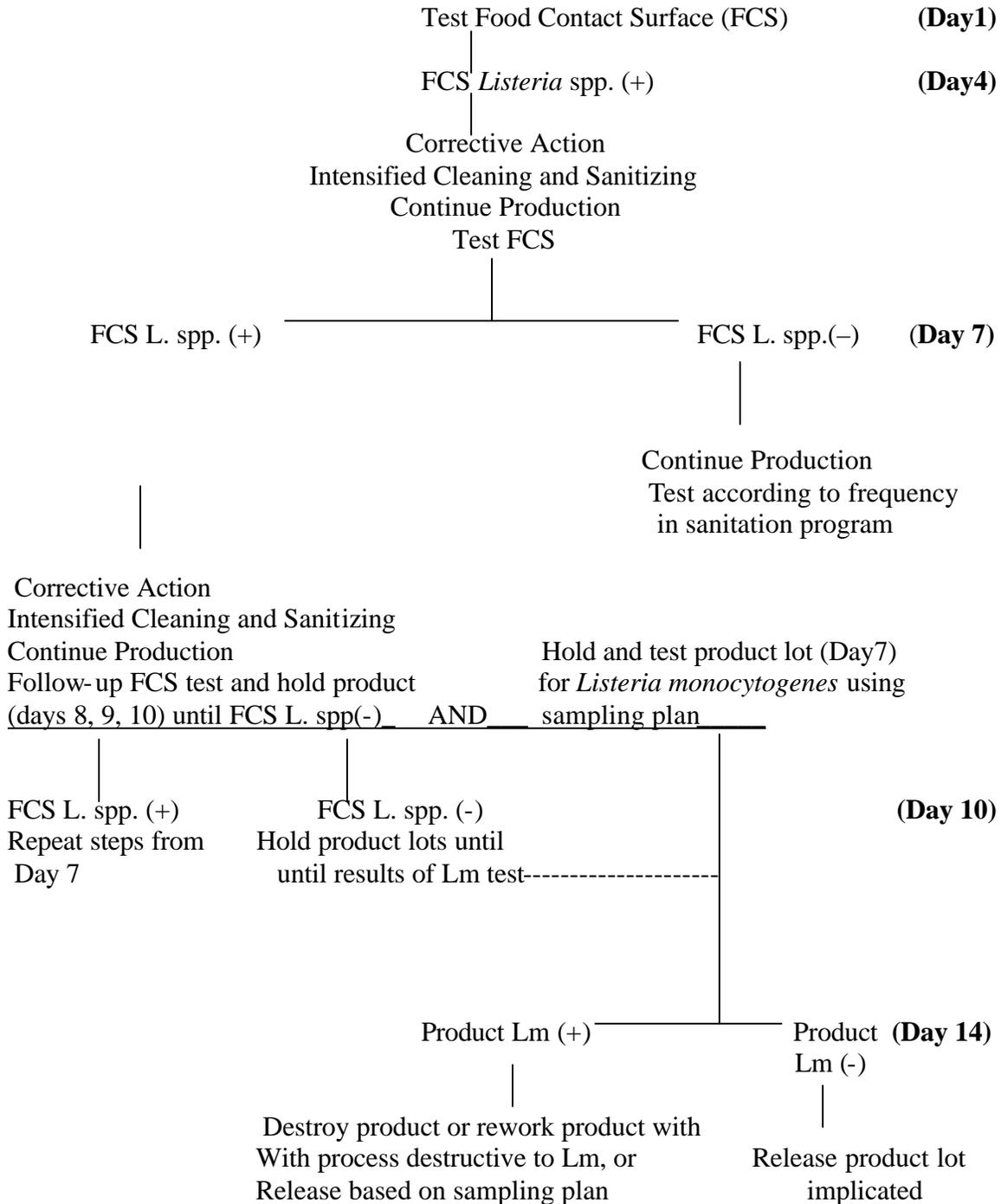
Case 13 $n=15, c=0$	Case 14 $n=30, c=0$	Case 15 $n=60, c=0$
Conditions of use reduce risk	Products with no growth due to antimicrobial or other formulation considerations such as pH, a_w , etc.	Products that support growth and that will be stored refrigerated
Example: Products with post-lethality treatments to reduce or eliminate <i>L. monocytogenes</i> ; e.g. those that received steam pasteurization or high pressure processing	Example: Products with antimicrobial agents or processes, e.g. hot dogs and deli meats with lactate or diacetate as additives	Example: hotdogs and deli meats that did not receive any post-lethality treatment or do not contain any antimicrobials

ATTACHMENT 6
HOLD-AND-TEST SCENARIO FLOWCHART

The following flow chart is a most likely scenario for a hold and test situation. The flowchart illustrates what an establishment could do in case of a positive food contact surface (FCS) test, and when a follow-up FCS test is positive. Establishments can design their own procedures or flowchart for their hold and test program. Repeated positive FCS test would imply an inadequate sanitation system or harborage of the pathogen and establishments should investigate and reassess their sanitation program, their equipment layout and design, product flow to determine the cause of the contamination.

This chart only addresses FCS testing with *Listeria* spp or *Listeria*-like organisms. If the establishment tests FCS for *L. monocytogenes* and result is positive, product in the sampled lot is considered adulterated. The establishment can destroy the product, rework the product with a process that is destructive of *L. monocytogenes*, or test product for *L. monocytogenes* and dispose of the product based on a sampling plan. In addition, the establishment must conduct follow-up testing starting from day 7 in the following chart.

HOLD-AND-TEST SCENARIO FLOWCHART



FCS: food contact surface

L spp.: *Listeria* spp. or *Listeria-like* organisms (test results available after 2 or 3 days)

Lm : *Listeria monocytogenes* (test results available after 6 or 7 days)

Enforcement strategy

Under 9 CFR 430, an establishment with deli and hot dog products in Alternative 3 must provide for testing of food contact surface (FCS). If the FCS tests positive for *L. monocytogenes* or *Listeria* Spp. or *Listeria*-like organisms, the establishment must conduct follow-up testing to verify its corrective actions. If during the follow-up testing another positive FCS occurs, the establishment must hold product lot implicated and test the FCS until the establishment corrects the problem as indicated by the test result. In addition, the establishment must test held product lots for *Listeria monocytogenes* using a sampling plan that will provide a statistical level of confidence. The flowchart above shows a test and hold scenario which an establishment in this type of situation can use. The following section describes the likely action and reaction of inspection personnel during a hold and test situation.

Day 1, 4

The testing program and the test results for food contact and non-food contact surfaces should be available to inspection program personnel. In case of a FCS testing positive for *L. spp.* or *Listeria-like* organism, inspection program personnel will verify that the establishment is performing the corrective actions as specified in the HACCP plan, Sanitation SOP or prerequisite programs, including any intensified cleaning and sanitizing. For deli and hot dog products in Alternative 3, inspection personnel should verify that the establishment is conducting follow-up testing for FCS to determine the effectiveness of the corrective actions, targeting most likely source of contamination and additional tests in surrounding FCS area, and recording all these.

Day 7

Results of the follow-up FCS tests are available on this day. If the FCS tests are negative, then the establishment continues with its normal production and sanitation program procedures. If the follow-up FCS tests are positive for *L. monocytogenes*, *Listeria* spp. or *Listeria*-like organisms, inspection program personnel should verify that the establishment is following its corrective action for a second FCS positive, including intensified cleaning and sanitizing. For deli and hotdog products in Alternative 3, inspection personnel should verify whether the establishment is holding the product produced that day and testing the product lot for *L. monocytogenes*. Inspection program personnel should verify whether the establishment is conducting follow-up testing of FCS during each production, and holding all products until a negative follow-up FCS test is obtained. Products produced on days 8, 9 and 10 are held because the follow-up FCS test is available after 3 days. The interim rule states that products must be held until the problem is corrected as indicated by testing. For establishments in Alternative 3 producing deli and hotdog products, inspection personnel can cite the establishment if these procedures are not followed.

Day 10

Inspection program personnel should verify that if the follow-up FCS test is positive, then production lots of deli and hotdog products in Alternative 3 corresponding to this FCS is

held and tested for *L. monocytogenes* and that the same procedures are followed as in the second FCS (+) test as in Day 7.

Day 14

For products that test positive for *L. monocytogenes*, inspection personnel should verify that the products are disposed properly, destroyed or reworked with a process destructive to Lm or released based on the sampling plan used, and that the product disposition is recorded accordingly.