2.2 Antimicrobial Agents (AMA) and Antimicrobial Processes (AMP)

According to the *Listeria* Rule, AMAs and AMPs must suppress or limit the growth of *Lm* throughout the shelf-life of the product. AMAs can include lactates and diacetates added in the formulation of the product and growth inhibitors added in the immediate packaging material. AMAs and AMPs must be included in the establishment's HACCP plan, **Sanitation Standard Operating Procedure (Sanitation SOP)**, or **prerequisite program** and the establishment must validate that the AMA or AMP is effective as used.

It is FSIS's expectation that AMAs or AMPs are designed to allow no more than 2-logs of growth of *Lm* over the shelf-life of the product. If the AMA or AMP is included in the establishment's HACCP plan, the establishment must validate and verify its effectiveness in accordance with 9 CFR 417.4. If the AMA or AMP is included in the establishment's Sanitation SOP, the effectiveness of the measures must be evaluated in accordance with 9 CFR 416.14. If the AMA or AMP is included in a prerequisite program other than a Sanitation SOP, the establishment must ensure that the program is effective and does not cause the hazard analysis or the HACCP plan to be inadequate. The establishment must include the program and the results produced by the program in the documentation that it maintains as required in 9 CFR 417.5(a). Expectations for the efficacy of AMAs are provided in Table 2.2. For further information on validation of AMAs and AMPs, see Appendix 2.1.

Question: Could cure (156 ppm added nitrite) be considered an AMA?

Answer: Sodium nitrite is primarily used to inhibit *Clostridium botulinum* growth and toxin production in cured meats. Studies have shown an inhibitory effect of nitrite, salt, and vacuum packaging on *Lm* growth in fish. The establishment would have to provide documentation on the inhibitory effect of nitrite on *Lm* in meat and poultry and indicate what other factors, such as salt concentration, are critical for the inhibitory effect.

1. Antimicrobial Agents (AMA)

AMAs are defined as substances added to RTE products that have the effect of suppressing or limiting growth of *Lm* in the product throughout the shelf life of the product (9 CFR 430.1). AMAs should allow no more than 2-logs of growth over the shelf life of the product. Examples of AMAs include: potassium lactate and sodium diacetate. Growth inhibition achieved by adding antimicrobials to product formulation depends on a variety of factors, such as:

1) The level of antimicrobial agent added,
2) pH of the product,
3) Moisture level of the product,
4) Product formulation, and
5) Whether the agent was added during formulation or to the finished product.

Some published studies on antimicrobials are reviewed in Attachment 2.2. **If establishments want to use such studies as part of their validation or support, they would need to identify all of the critical operation parameters in the study and apply them to their process.** See the section below on documenting the effectiveness of AMAs and AMPs and Appendix 2.1 for more information.

According to the *Listeria* Rule, the AMA or AMP must be effective throughout the shelf life of the product (9 CFR 430.1). The shelf life of the product is defined as the amount of time the product can be stored under specified conditions and still remain safe with acceptable quality. A report

slaughter of livestock and poultry, and processing of meat, poultry, and egg products. FSIS evaluates whether new technology and new ingredients affect product safety, inspection procedures, inspection program personnel safety, or if they would require the waiver of a regulation.

Substances used as new technology or new ingredients must also meet the requirements for safety and suitability under the Agency's food ingredient approval process. While FDA has the responsibility for determining the safety of food ingredients and additives, as well as prescribing safe use, FSIS has the authority to determine that new ingredients and new uses of ingredients are suitable for use in meat, poultry, and egg products.

FDA and FSIS have a Memorandum of Understanding (MOU) regarding the review, approval, and listing of food ingredients and sources of radiation used in the production of meat, poultry, and egg products. This agreement establishes the working relationship to be followed by FSIS and FDA in responding to requests for the sanctioning of the use of food ingredients and sources of radiation subject to regulation by FDA and intended for use in the production of meat, poultry, and egg products. This review is normally done simultaneously by both agencies. The MOU information can be found at:
http://www.fsis.usda.gov/Regulations_&_Policies/Labeling_FDA_MOU/index.asp

The FSIS Innovations (New Technology) Staff reviews new technology and new ingredients that can be applied in meat, poultry, and egg processing to facilitate the introduction of the new technology in establishment or plant operations. New technology and new ingredients for use on post-lethality RTE meat, poultry, and egg products to control the growth of *Lm* should be sent to this office for review. FSIS issued the document "Guidance Procedures for Notification and Protocol Submission of New Technology" to aid in the submission of applications for review of new technology and new technologies by FSIS. Those to which FSIS has "no objection" to their use in FSIS establishments are posted on the FSIS website at:
http://www.fsis.usda.gov/Regulations_&_Policies/New_Technologies/index.asp

A listing of ingredients that have been reviewed and approved by FDA and FSIS are available in 9 CFR Part 424, Subpart C, 424.21 "Use of food ingredients and sources of radiation" found at http://edocket.access.gpo.gov/cfr_2010/janqtr/9cfr424.21.htm. This regulatory listing of approved ingredients is now updated quarterly through revisions of FSIS Directive 7120.1"Safe and Suitable Ingredients Used in the Production of Meat, Poultry, and Egg Products" to expedite the posting of new approved substances. It is available at:
http://www.fsis.usda.gov/About_FSIS/labeling_&_consumer_protection/index.asp.

The above technology and ingredient reference resources should be used when considering the use of a technology or ingredient.

2.7 Glossary

Antimicrobial Agent (AMA): A substance in or added to an RTE product that has the effect of reducing or eliminating a microorganism, including a pathogen such as *Lm*, or that has the effect of suppressing or limiting growth of a pathogen, such as *Lm*, in the product throughout the shelf life of the product. Examples include potassium lactate and sodium diacetate, both of which limit the growth of *Lm* (9 CFR430.1).

Antimicrobial Process (AMP): An operation, such as freezing, applied to an RTE product that has the effect of suppressing or limiting the growth of a microorganism, such as *Lm*, in the product throughout the shelf life of the product (9CFR 430.1).

Log Reduction: A 90% reduction of a pathogen. For example, a 2-log_{10} reduction is a 99% reduction of a pathogen.

Post-lethality Treatment (PLT): A lethality treatment that is applied or is effective after post-lethality exposure. It is applied to the final product or sealed package of product in order to reduce or eliminate the level of pathogens resulting from contamination from post-lethality exposure (9 CFR 430.1).

Prerequisite Program: A procedure or set of procedures that is designed to provide basic environmental or operating conditions necessary for the production of safe, wholesome food. It is called "prerequisite" because it is considered by scientific experts to be prerequisite to a HACCP plan (9 CFR 430.1).

Sanitation Standard Operating Procedure (Sanitation SOP): Written procedures for sanitation that describe all of the procedures the establishment will perform daily, before, and during operations, sufficient to prevent direct contamination or adulteration of products, according to 9 CFR 416.12(a).

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Attachment 2.1: Post-Lethality Treatments

NOTE: 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 $\log_{10}$ reduction of *Lm* in inoculated vacuum packaged fully cooked sliced chicken. The reduction was effective when single–packaged breast fillets, 227 gm-package strips, and 454 gm-packaged strips were heat treated at 90º 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 *Lm*.

Information gathered from the summary or abstract:

Post-lethality treatment: hot water pasteurization or steam pasteurization
Products: fully cooked chicken breast fillets and strips
Procedure: fully cooked products were surface inoculated with *Lm*, vacuum packaged and pasteurized
Equipment used for the pasteurization treatment:
Steam pasteurization: pilot-scale steam cooker
Hot water pasteurization: pilot-scale hot water cooker
Temperature of pasteurization: 90°C
Reduction of *Lm*: 7-log reduction
Products and time of pasteurization that resulted in 7-log reduction

Product	**Time of pasteurization (min)**
Single-packaged breast fillets	5
227g-package strips	25
454 g-packaged strips	35

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

Pre-package surface pasteurization treatment of fully cooked meat removed from its packaging wrap and inoculated with *Lm* resulted in a 1.25 to 3.5-log reduction with a treatment time of 60-120 sec at 475 to 750º 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. 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 to 4.3-log reduction for bologna, or a 2.0 to 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 to submerge cooked RTE deli-style whole or formed turkey, ham and roast beef, removed from their package, inoculated with *Lm* and vacuum packaged. Results show a 2 to 4-log decrease in the levels of *Lm* in inoculated products post-cooked at 195-205º F for 2-10 min.

Treatment of processed foods with acidified sodium chloride (ASC) is another example of pre-packaging treatment. ASC is an antimicrobial agent that is approved for use on processed meat food products (unless precluded by standards of identity in 9 CFR 319), prior to packaging of the food for commercial purposes (21 CFR 173.325(f)). It is applied as a dip or spray at levels that result in a sodium chlorite concentration of 500 to 1,200 ppm in combination with any GRAS acid at levels sufficient to achieve a pH of 2.5 to 2.9. It is approved as a secondary direct food additive and considered as a processing aid, with very temporary or short term technical effect (bactericidal antimicrobial activity), after which it rapidly degrades to leave no long term residues or actives remaining (Kemp, Alcide Corp., personal communication, 2003). Because of this, it does not have to be included in the ingredient listing of the label. Marsden et al. (2000, unpublished), evaluated sodium chlorite (1,200 ppm) with 0.9% citric acid for its effectiveness in reducing *Lm* on retail sausages. Results show that a water wash gave a 1.2-log reduction of *Lm*. An ASC dip for 15 sec provided a 1.0-log reduction better compared to water wash. ASC exposure time of 30 sec gave 1.1 and 1.6-log reductions over the water wash control, for spraying and dipping, respectively. Spray wash or dipping was found to be comparable in antibacterial effectiveness against *Lm*.

II. High-Pressure Processing

High-pressure processing (HPP) is a technology that subjects food to elevated pressures, with or without the addition of heat, to inactivate microorganisms and extend microbiological shelf life. This technology provides a means of ensuring food safety for those products that are difficult to heat treat 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 HPP in inactivating *Lm* 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 *Lm* cocktail in these 3 products and HPP treatment at 87,000 psi for 3 minutes showed no recovery of *Lm* after 61 days of storage at 34° F. No pressure-injured cells were detected. No adverse organoleptic effects were 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.

Attachment 2.2: Antimicrobial Agents or Processes

I. Use of Antimicrobial Ingredients including Bacteriophages, Lactates, Acetates, Diacetates, and Ozone

Bacteriophages are viruses that infect bacteria, and cause cell death. Bacteriophage preparations may be sprayed on RTE products to reduce or eliminate *Lm*. These preparations (a mixture of equal proportions of six different individually purified lytic-type bacteriophages specific against *Lm*) are applied as a spray at a level not to exceed 1 ml of the additive per 500 cm^2 product surface area.

Guenther et al., (2009) showed that *Lm* pathogen-specific bacteriophages could reduce bacterial counts by up to 5 logs when applied to the surface of hot dogs (sausages) and sliced turkey breast (cold cuts).

Ozone is an antimicrobial gas usually applied in an aqueous solution to products, food contact surfaces as a continuous spray (e.g., belts, moving tables), and non food contact environmental surfaces. Currently, the use of ozone is permitted by FDA and FSIS (21 CFR 173.368, FSIS Directive 7120.1) for use with all meat and poultry products, including RTE meat and poultry products.

Buege et al., (2004) showed 1.0 to 2.4 log reductions (average 1.5) of *Lm* when 0.6 ppm ozone for 30 seconds was applied to ham, salami, meatloaf, natural casing wieners, and skinless wieners.

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 *Lm*. These antimicrobials inhibit growth of pathogens by inhibiting their metabolic activities.

Seman et al., (2002) developed a mathematical model capable of predicting the growth or stasis of *Lm* 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 *Lm*.

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 *Lm* by applying it to the surface of 100g of cured meat (four slices).

Sodium chloride content was found to have a negative correlation to growth rate. The investigators provided a final regression equation predicting the growth of *Lm* 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 it with actual *Lm* 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 *Lm* exceeded those of the observed values by about 24%.

The study also emphasized the importance of **moisture content** in the application of lactates and diacetates as antimicrobial agents. The article reports that **"The results show that increasing amounts of potassium lactate syrup and sodium diacetate decreased the growth rate of *Lm*, 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.** 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 *Lm*. The calculations would also require knowledge of the finished product sodium chloride and moisture contents."

Recall Alert

An investigation of a 2007 recall of RTE cooked chicken products contaminated with *Lm* showed that the establishment failed to maintain sanitation, and antimicrobial agent failed to suppress *Lm*. The moisture levels were higher in the product than in the establishment's supporting documentation, which could have allowed *Lm* growth.

Table 2 from the study shows that different finished product moisture levels, amount of sodium chloride, and lactate and diacetate result in different levels of *Lm* growth rate.

% salt	% sodium diacetate	% potassium lactate syrup	% product moisture	*Lm* growth rate (wk^{-1})
1.50	0.15	7.0	74.0	0.0
1.50	0.05	2.5	74.0	0.0991
2.20	0.20	4.75	64.5	0.0
2.20	0.10	0.25	64.5	0.1338

The investigators advised that this validated model is specific to the products designed for the study and the *Lm* 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 (Seman et. al.) 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 *Lm*. 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 *Lm* 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 is a unique tool used to calculate the levels of lactate and diacetate required to retard the growth of *Lm* 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 includes:

- Instructions on how to use the model,
- Explanation on the development of the model,
- Information on the anti-microbial effects of lactate and diacetate,

- Lactates and diacetates and use of these products,
- Regulations and labeling, and
- Literature references.

The model can be accessed by visiting the Purac website at: http://www.purac.com/EN/Food/ingredients/Meat_poultry_and_fish/Preservation/Food-safety/Listeria.aspx

Bedie et al., (2001) evaluated the use of antimicrobials, including in frankfurter formulations, on *Lm* populations during refrigerated storage. Fully cooked and cooled frankfurters were inoculated with 10^3 to 10^4 CFU /cm^2 of *Lm* 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 (%)	*Lm* 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 zero increase in the number of inoculated *Lm* cells (bacteriostatic), while reduced pathogen growth refers to a decrease in the number of inoculated *Lm* cells (bactericidal) in the product. In this study, tables showed that 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 *Lm* up to 70 days at refrigerated storage of 4° C. If the lethality treatment is adequate to eliminate *Lm*, then the only probable source of *Lm* 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 *Lm* 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.

A 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 sec. Storage at 4° C shows:

Treatment	Levels (%)	*Lm* Growth Inhibition
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 + Glucuno-delta-lactone	1.8 0.25	120 days no growth, 35-50 days growth 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 *Lm* mixture on multiple areas of the surface of each link. Packages were vacuum-sealed and stored at 4.5º 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 secs, 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 *Lm*. Results are as follows:

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

A 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 *Lm*. The packages were vacuum-sealed to 95 kPa and incubated at 4 and 10° C.

Results show that the addition of 2% or 3% potassium lactate in frankfurters can appreciably enhance safety by inhibiting or delaying the growth of *Lm* 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	*Lm* 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 to control the risk of *Lm* contamination of RTE meat and poultry products.

Studies on meat formulations for hotdogs using NOJAX® AL™ showed that the use of the casings provide a lethality hurdle to the growth of *Lm*, not just an inhibitory effect. The lethality impact is delivered within the first hours/days of the sausage/hotdog package life. This impact is dependent on many variables, but is generally in the range of 1 – 2 log decrease of *Lm* at high levels of inoculation. This performance has been observed in challenge studies conducted on hotdogs 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 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 *Lm* begin to grow.

NOJAX AL is available in the U.S., and has been approved 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 Program Delivery Division. 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 60-90 days, with a not to exceed temperature of 85º F.

This technology can be applied to most hotdogs and sausages that are encased in cellulose casing. This casing intervention can be used in any instance were 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 or plant reconfigurations; 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 hotdogs.

Janes et al., (2002) investigated the effect of nisin added to zein film coatings (Z) coated onto cooked RTE chicken against *Lm*. Cooked chicken samples inoculated with *Lm* 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 days at 4°C, *Lm* 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 *Lm* on the surface of RTE chicken was found when using edible zein film coatings containing nisin at a storage temperature of 4°C.

A processing plant would use film coatings by fully processing the meat products, then coating 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. The study by Janes et. al. 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 *Lm* when used in combination than when used singly.

- These antimicrobials (described in the guideline) 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 listeriostatic 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 establishment should verify the effectiveness of the antimicrobials used in these studies for other processed meat products and other storage temperatures.

- Antimicrobials used in the formulation should 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. 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.

- These antimicrobials may be a cost-effective antilisterial method that very small establishments can use.

Appendix 2.1 Validation

I. Validation
II. Scientific Support
1. Published Processing Guidelines
2. Scientific Articles from a Peer-Reviewed Journal
3. Challenge or Inoculated-Pack Studies
4. Validated Predictive Microbial-Modeling Programs
5. Establishing the Shelf-life of the Product
III. In-plant Demonstration
IV. Validation Examples

I. Validation

Validation is the process of demonstrating that the HACCP system as designed can adequately control identified hazards to produce a safe, unadulterated product. **There are two distinct elements to validation:**

1) **The scientific or technical support for the HACCP system (design).** This consists of having scientific and technical documentation that demonstrates that the designed process can control the identified hazard. In other words, will the HACCP **work in theory**?

2) **The initial practical in-plant demonstration proving the HACCP system can perform as expected (execution)**. This consists of having records that demonstrate that the HACCP plan achieves what it is expected to achieve. In other words, **does the plan work in practice?**

Validation encompasses activities that make up the entire HACCP system. Validation is an important component to the development of a HACCP system but has particular importance for products produced under the *Listeria* Rule. Validation, as it relates to the requirements in the *Listeria* Rule, will be covered in this Appendix. In particular, considerations for scientific support and in-plant data for AMAs, AMPs, and PLTs will be covered. Further recommendations can be found in the complete Validation guidance [http://www.fsis.usda.gov/PDF/HACCP_Systems_Validation_Draft_Guidance_0412.pdf].

Question: Can establishments use the studies cited in the Compliance Guidelines for validation as they use the Compliance Guidelines in Appendices A and B in the Final Rule for certain meat and poultry products to validate cooking and cooling (stabilization) processes?

Answer: Yes, provided the product, processing procedures, and ingredients are equivalent to those in the studies. For example, if the pH and concentration of antimicrobial in the study were both considered critical, then the product must have that pH and contain the antimicrobial in the concentration used in the study.

II. Scientific Support

The **first element of validation is scientific support** (design). There are several types of scientific support that would be considered acceptable for validating an AMA, AMP, PLT, or other treatment. These include:

- Published processing guidelines

lactate/diacetate to be used and the log suppression of *Lm* based on the information provided.

- Growth models on the use of antimicrobial agents are available mostly for cured products. For uncured products where there are no growth models, validation studies need to be conducted per product.

- Verify the effectiveness of the antimicrobial agent/process used by testing for *Lm* growth during the shelf life of the product, at a certain frequency.

- Maintain and monitor records of validation, verification, and corrective actions for deviations from the effective application of antimicrobial agents/processes.

5. Establishing the Shelf-life of the Product

As stated in Section 2.2, the AMA or AMP must be effective throughout the shelf life of the product (9 CFR 430.1). The shelf life of the product is defined as the amount of time the product can be stored under specified conditions and still remain safe with acceptable quality. In order to demonstrate effectiveness of control measures over the shelf life of the product, the establishment would need to establish their expected shelf life through a challenge study, shelf-life study, or other supporting documentation such as predictive microbial modeling. This study or other supporting documentation should demonstrate that the AMA or AMP is effective in controlling growth over the product's shelf life. **Although establishments are not required to label their product with a "use-by" date, or other information indicating the shelf life of the product, a prudent establishment would use this labeling to help ensure that the product is not consumed after the shelf life is complete.**

An establishment may perform the shelf-life study or provide other supporting documentation establishing the shelf life of the product. A shelf-life study is one that measures the increase or decrease in the number of the target organism or pathogen during storage. For an AMA or AMP, a shelf-life study is important to perform as part of the challenge study, because it determines the time (in days) the growth of *Lm* is controlled. Both refrigeration temperatures (e.g., 40°F) and a slightly abusive temperature (e.g., 45°F) should be used in the shelf-life study in order to ensure that if *Lm* is present and viable, growth will occur and can be measured throughout shelf life. This slightly abusive temperature also represents the worse-case conditions that could occur during cold-chain storage and handling.

Some of the factors that should be considered in the shelf life study of a product with an added AMA to determine that the agent is effective in suppressing growth of *Lm* are:

1. Suppression of *Lm* growth in product during shelf life – growth should be lower in the product with added antimicrobial than growth in the untreated control. Although the Compliance Guidelines set a maximum of less than 2 log growth of *Lm* during the shelf life of product with added antimicrobials for the purposes of the challenge study, it is best to target a lower amount of growth than this.

2. The rate of growth of *Lm* in product-- the *Lm* growth-rate in product with added antimicrobial should be slower than the growth rate in product without added antimicrobial.

3. Temperature for holding product during the shelf life study – Most studies use the temperature that the product is normally held during storage as the temperature during shelf life

studies e.g., refrigerated temperature of 38-40 ° F. Shelf life studies can also use or include a temperature of 45 ° F to hold product since this reflects consumer handling.

A resource article for conducting challenge studies for validation of antimicrobial agents is the Considerations for Establishing Safety-Based Consume-by Date Labels for Refrigerated RTE Foods (NACMCF, 2004), found at:
http://www.fsis.usda.gov/ophs/nacmcf/2004/NACMCF_Safety-based_Date_Labels_082704.pdf. This article gives guidance on how to determine the shelf-life of a RTE product containing an added antimicrobial agent that is supposed to suppress *Lm* growth during the refrigerated shelf-life. Most studies use the temperature which the product is normally held during storage as the temperature during shelf life studies, e.g., refrigerated temperature of 38-40° F. As described above, shelf-life studies also should use or include a temperature of 45° F which reflects consumer handling. The NACMCF document recommended to using a higher temperature for shelf-life studies because foods can encounter a range of temperatures below and above 45° F, with higher temperatures more likely in grocery store cases and during consumer handling. Therefore these temperatures more accurately reflect reality.

NOTE: A product with an added antimicrobial agent demonstrating *Lm* growth of <2 log at a storage temperature of 38-40° F and at 45° F or above would be viewed by FSIS as more protective of public health than another product showing the same growth only when stored at 38-40° F.

III. In-Plant Demonstration Data

The **second element of HACCP systems validation is initial in-plant validation** which may include in-plant observations, measurements, microbiological test results, or other information demonstrating that the *Lm* control measures, as written into a HACCP system, can be executed within a particular establishment to achieve the process's intended result.

As of the date of this guideline, FSIS realizes that some establishments may not have kept their initial in-plant demonstration documents from when HACCP was originally implemented. Those establishments that have not will be allowed the time to assemble their in-plant demonstration documents. The Agency will describe and explain these documents in a future Federal Register Notice that it intends to issue when it finalizes the Compliance Guideline on HACCP systems validation. Until the Federal Register Notice issues and further instructions are given to FSIS personnel, FSIS will not cite the lack of in-plant validation data as the only reason for the documentation of noncompliance.

In cases where the process specifications described in the supporting documentation are implemented in the same or similar enough way (see box below) in the establishment's process, and when the scientific supporting documentation used contains microbiological data specifying the level of pathogen reduction achieved by the intervention strategy for the target pathogen identified in the hazard analysis, the establishment should:

- Identify the critical operating parameters in the scientific support, AND
- Translate them in the HACCP system, AND
- Demonstrate that the critical operating parameters are being met by gathering 90 days of execution data.

> Implementing process specifications in a similar enough way in the establishment's process means that changes among the critical operational parameters used in the scientific support and those used in the actual process will not affect the efficacy of the AMA, AMP, PLT, or other treatment. Generally, establishments should use the same critical operational parameters as those in the support documents. In some circumstances, establishments may be able to support using critical operational parameters that are different from those in the support documents (e.g., higher concentrations of antimicrobials or higher thermal processing temperatures). In these cases, establishments should provide justification supporting that the levels chosen are at least as effective as those in the support documents. In addition to ensuring that the levels chosen are at least equally as effective, establishments should also ensure the levels are also safe and suitable per FSIS Directive 7120.1.

By demonstrating that the critical operating parameters are being met through the collection of execution data, the establishment will have addressed the second element of validation – in-plant demonstration data without the need for further microbiological data. In cases where the process specifications described in the supporting documentation **are not implemented** in the same or similar enough way in the establishment's process, or when the scientific supporting documentation used **does not contain** microbiological data specifying the level of pathogen reduction achieved by the intervention strategy for the target pathogen identified in the hazard analysis, the establishment should:

- Validate that the intervention as modified actually achieves the effect documented in the scientific supporting documentation (Element 1), AND
- Validate that the modified critical operating parameters are being met, AND
- Validate the intervention's effectiveness under actual in-plant conditions.

> **NOTE**: Microbiological data (e.g., challenge studies or in-plant data) is encouraged but not required to comply with the minimum initial validation requirements provided the establishment has adequate scientific supporting documentation (the first element of validation), is following the parameters in the scientific support, and can demonstrate that it can meet the critical parameters during operation (the second element of validation). In order to meet the second element of validation (in-plant demonstration data) the establishment would need to gather data (such as monitoring records of water temperature for a hot water pasteurization process or of water activity resulting from a drying process) over the initial 90 days demonstrating the critical operational parameters are being achieved.

The establishment should develop the appropriate execution data during the initial 90 days of implementing a new HACCP system, or whenever a new or modified food safety hazard control is introduced into an existing HACCP system as identified during a reassessment. During these 90 calendar days, an establishment gathers the necessary execution data to demonstrate critical operating parameters are being achieved. In essence, the establishment would repeatedly test the adequacy of the process steps in the HACCP system to establish that the HACCP system meets the designed parameters and achieves the intended result as described

in the HACCP Final Rule. These execution data become part of the validation supporting documentation along with the scientific support used to design the HACCP system.

For examples of the type of scientific support and in-plant demonstration data that would be expected for different types of *Lm* controls, please see the validation examples taken from the FSIS Compliance Guideline on HACCP Systems Validation on the following pages.

Appendix 2.2: Sanitation

I. Introduction
II. Pre Operational Sanitation Procedures
III. Operational Sanitation Procedures
  1. Controlling Temperature and Air Handling Units
  2. Equipment Design
  3. Traffic Control
  4. Employee Hygiene
  5. Controlling Cross Contamination
IV. Sanitation During Construction
V. Intensified Sanitation in Response to Positives
VI. Determining the Effectiveness of the Sanitation Program

I. Introduction

The cornerstone of the *Listeria* Rule is sanitation within the post-lethality environment. All other layers of antimicrobial interventions (antimicrobial agents, post-lethality treatments, antimicrobial processes) are built upon the effective design of the establishment's sanitation program to control *Lm* and **will not** be effective if the sanitation program is poorly designed.

Understanding the growth/survival characteristics is critical to the success of controlling the pathogen. *Lm* is more heat-resistant than most foodborne pathogens. It can survive freezing and drying. *Lm* resists high salt levels, nitrite, and acid and can grow in vacuum packaged products. Most importantly, the pathogen can grow in a damp, cool environment. Once the bacteria attaches to a surface it can form a biofilm and establish a niche, or harborage site, which can become more resistant to superficial cleaning regimens. Bacteria can then spread from the niches to food-contact surfaces and product.

The critical components of an effective sanitation program to control *Lm* can be divided into the following major categories. These include:

- Pre-operational cleaning and sanitizing procedures that are effective in preventing *Lm* from forming niches or harborage sites in the processing environment.

- Operational sanitation procedures to prevent cross-contamination in the RTE processing environment.

- Intensified cleaning and sanitizing procedures in response to positive sampling results.

- Documentation and verification of cleaning and sanitizing procedures.

Establishments are required to develop and implement the Sanitation SOP regulatory requirements, 9 CFR 416.12 through 416.16. Proper and effective sanitation involves both cleaning and sanitizing, and verifying that the cleaning and sanitizing were effective. This involves developing and implementing written sanitation standard operating procedures (Sanitation SOPs). Sanitation SOPs could be viewed as the first step to designing a total system, including the HACCP plan that will prevent, eliminate, or reduce the likelihood of pathogenic bacteria from entering and harboring in the plant environment.

Sanitizers

Cleaning and sanitizing are vital to any effective sanitation program. Thorough cleaning should be followed by sanitizing. Generally, the cleaning step is to remove all waste materials and soils, and the sanitizing step is to destroy all microorganisms. Careful consideration should be given to selecting both cleaning and sanitizing solutions. It is important to use solutions that are compatible with the equipment materials, such as stainless steel or heavy plastics, and solutions that are effective in destroying the type of bacteria commonly associated with the type of products produced in the establishment.

Rather than relying on a single sanitizer, **rotating** sanitizers will help prevent the development of microorganisms resistant to a particular sanitizer. The concentration and application processes for all sanitizers approved for use in meat and poultry establishments are referenced in Title 21 Code of Federal Regulations (21 CFR), Part 178, section 178.1010. All cleaners and sanitizers commercially available should have, at the minimum, the following information either on the label or available on a specification sheet that must accompany the product:

- Product Description
- ** **Instructions on how to use the product (concentration, method of application, contact time, temperature)**
- Properties
- Safety Information

Additional information that is sometimes available includes:

- Benefits
- Quality Assurance Statements

**Effectiveness against *Listeria*.

Some manufacturers provide labeling in both English and Spanish, which makes the products more user friendly in various environments. At least one manufacturer also has commercially available color coded products that are easy to associate with a particular cleaning or sanitizing task.

Recommendations for sanitizers inactivating *Lm* in biofilms on stainless and plastic conveyor belts:

- Chlorine and iodophors are not effective inactivating *Lm* in biofilims on stainless steel.
- The most effective sanitizers are acidic (not neutral) quaternary ammonium compounds, peracetic acid, and chlorine dioxide.
- The less effective are the mixed halogens and acid anionics sanitizers, which were less effective than the sanitizers listed in #2.
- And the least effective sanitizers were chlorine, iodophors, and neutral quaternary ammonium compounds.

III. Operational Sanitation Procedures to Prevent Cross Contamination Between Raw and RTE Post-Lethality Environment

1. Controlling Temperature and air handling units

- Maintain temperature in processing areas and packaging rooms as stated in the HACCP plan, Sanitation SOPs, or Prerequisite Programs.
    - Maintain cold temperature (<50º F) in packaging room for products that are to be refrigerated or frozen, as stated in the HACCP plan, Sanitation SOPs, or Prerequisite Programs to prevent *Lm* growth in the RTE processing environment.
    - Monitor temperatures as stated in the HACCP plan, Sanitation SOPs, or Prerequisite Programs.
- Establish positive air pressure movement out of the RTE room into the raw processing areas.
- Clean cooling units and air handling units at some specific frequency.
- Immediately address and correct problems of dripping condensation and standing water. Production of RTE products should be stopped during repairs and corrective actions for these problems. The equipment and processing area should be cleaned and sanitized after all the repairs and corrective actions are finished.

2. Equipment Design

- Evaluate the equipment to ensure that it can be easily dismantled for cleaning and is durable.
- Investigate for potential *Lm* harborage sites, such as hollow rollers.
- If new equipment is purchased, select equipment designed to enhance cleaning
    - All areas and parts should be accessible for manual cleaning and inspection or be readily disassembled.
    - Closed conveyor designs are more difficult to clean. Equipment on the processing line should be as easy to clean as possible.
    - Avoid hollow conveyor rollers and hollow framing. If hollow material is used, have a continuous weld seal instead of caulk.
    - Select food contact surfaces that are inert, smooth, and non-porous.
    - Equipment should be self-draining or self-emptying.
- Maintain equipment and machinery by adopting a regular preventive maintenance schedule (QA should verify performance)

    - Damaged, pitted, corroded, and cracked equipment should be repaired or replaced.

    - Repair parts or machinery in a manner that prevents food deposits that are not easily removed with normal cleaning.

    - Use separate tools for RTE equipment only. Sanitize them before and after each use.

    - If compressed air is used, maintain and replace in-line filters regularly.

    - Use lubricants that contain listericidal additives, such as sodium benzoate. *Lm* can grow in lubricants that are contaminated with food particles.

  - Clean maintenance tools (including wrenches, screws, and tool boxes) on a regular basis. Consider designating certain tools for raw and RTE areas.

3. Traffic Control

One critical component of an effective sanitation program is control of the movement of personnel and raw product to prevent cross-contamination of RTE finished product and FCSs within the post-lethality environment. Establishments should examine product routes from heat treatment or other antimicrobial control steps to eliminate *Lm,* to final packaging. The following are steps that can be used to develop control procedures.

Establish traffic patterns to eliminate movement of personnel, meat containers, meat, ingredients, pallets, and refuse containers between raw and finished product areas. If possible, employees should not work in both raw and RTE areas. If they must work in both areas, they must change outer and other soiled clothing, wash and sanitize hands, and clean and sanitize footwear.

- If possible, use air locks or vestibules between raw and RTE areas.

- Use foam sanitizing spray systems on either side of the RTE room door on a timed system or triggered by entry/exit.

- Clean, dry floors are preferable to foot baths at the point of entry because effective concentrations of disinfectant are difficult to maintain and may become a source of contamination.

  - If foot baths are absolutely necessary:

    - Wear rubber or other non-porous boots.

    - Maintain them properly, so that they are clean and maintain effective levels of sanitizer.

    - Solutions should contain stronger concentrations of sanitizer than normally used on equipment (e.g., 200 ppm iodophor, 400-800 ppm quaternary ammonia compound).

    - Use a minimum depth of 2 inches.

NOTE: Chlorine is NOT recommended for foot baths because of rapid inactivation, especially if cleated boots are used. The accumulation of biological material adhering to the cleats inactivates (or reduces) the bioavailability of chlorine, making it less effective. Monitor and maintain the strength of the chlorine solution, if used.

4. Employee Hygiene

Development of employee hygiene procedures to prevent the contamination of FCSs should be the responsibility of management. The employee should be responsible for preventing contamination of food products and the management should be responsible for ensuring that the employee is properly trained and maintains good practices.

- Employee responsibilities and actions should include:
    - Using a 20 second hand wash, allowing the soap suds to be in contact with the hands for this period of time, after using restroom facilities.
    - Washing hands before entering the work area, when leaving work area, and before handling product.
    - If gloves are worn:
        - Gloves that handle RTE product should be disposable.
        - Dispose immediately and replace if anything other than product and FCS is touched.
        - Dispose of gloves when leaving the processing line.
    - Remove coats, gloves, sleeves and other outer clothing when leaving RTE areas.
    - Do not wear coats, gloves, sleeves or other outer clothing inside restrooms or cafeterias.
    - Do not store soiled garments in lockers.
    - Do not eat in the locker room or store food in lockers because food may attract insects and vermin.
    - Do not store operator hand tools in personal lockers. This equipment must remain in the RTE area at all times.
    - Do not allow employees who clean utensils and equipment for raw materials to clean RTE utensils and equipment, if possible.
    - The tools to clean utensils and equipment for raw materials must be different than those used to clean RTE utensils and equipment. In either case, the intent is to prevent cross contamination of finished product.
    - Management responsibilities should include:

    - Providing hand washing facilities at proper locations.

    - Ensuring that the employee receives proper hygiene instruction before starting – use of hand soaps and sanitizers, no-touch dispensing systems, and boot and doorway sanitizing systems.

    - Developing a system for monitoring employee hygiene practices.

    - Developing a system for tracking the training, testing, and certification.

    - Retraining employees before placing them back into production if they are absent from the job or have failed to follow acceptable hygiene practices. This will help ensure that the employees are following current, acceptable hygiene habits.

- Do not permit maintenance employees in RTE areas during operations if possible, primarily because they may cause direct product contamination or adulteration if they touch or lay their "dirty" equipment hands onto food contact surfaces. If this is not possible:

    - Consider the need to cease operations until a full cleaning and sanitizing is done, or,

    - Require maintenance personnel to change outer clothing and any other soiled clothing, use separate tools for raw and RTE areas (or wash and sanitize tools and hands prior to entering RTE areas) and wear only freshly cleaned/sanitized footwear in such areas.

    - Use separate equipment, maintenance tools and utensils for the RTE and raw areas. If not possible, there should be a time separation between raw processing/handling and RTE processing in order prevent cross contamination of finished product.

5. Controlling Cross Contamination

- For establishments processing RTE products, establish procedures to ensure that other non-meat or non-poultry RTE ingredients do not cause cross-contamination with *Listeria*.

- Maintain an effective rodent and insect infestation preventive and control program. Rats, mice, and insects are sources of *Listeria* and other microbial contamination.

- Eliminate standing water which can facilitate the spread of *Lm* into other areas of the plant. Sanitizer boluses can be used to sanitize standing water on a continuing basis.

- Discard products that touch environmental surfaces, such as products falling on the floor, if the product cannot be properly re-conditioned (e.g., by washing).

- Pallets can serve as a source of cross-contamination – pallets for raw materials should not be used in RTE areas or used for finished product.

- Do not allow condensation to build up or drip over exposed RTE product.
- Do not spray high pressure hoses near exposed product. Aerosols could develop that could contaminate the product.
- Do not allow employees to store knives, gloves, or equipment in their lockers. Provide designated storage areas for these items.
- Employees should not wear gloves, coats, or aprons in the restroom or break areas.
- Drains from the "dirty" or "raw" side should not be connected to those on the "clean" or "cooked" side.

Dual Jurisdiction Establishments

Because FSIS-regulated products are susceptible to *Lm* outgrowth:

It is advisable, due to the food safety nature of FSIS-regulated product, to separate processing areas for FSIS-regulated products and FDA-regulated products by time or space, such as scheduling processing on different days. If that is not possible, schedule FSIS product processing first, then FDA product processing. If FDA product is produced first, a complete clean-up and sanitizing before starting FSIS product processing is required.

Because of the risk for cross contamination, consider assigning different personnel for FSIS and FDA products and processing areas, if possible, especially if both are conducted on the same day. If not possible, have personnel clean hands thoroughly, and use unused, clean coats, new gloves and hairnets, and sanitized boots for FSIS and FDA processing.

IV. Sanitation During Construction

Dust generated by construction activities can move throughout the plant on air currents or be transferred by people or equipment traveling through the construction area into other areas of the establishment. A study by De Roin et al., (2003) showed that *Lm* in dust can survive and grow, once in contact with meat surfaces. Construction or maintenance activities that can result in *Lm* contamination of RTE product of FCS include removal of drains, removal of floor coatings, removal of a wall or ceiling that has absorbed moisture, movement of potentially contaminated materials through RTE areas or areas that directly connect with RTE processing areas, and exposure of areas typically not accessible for cleaning. Tompkin (2002) considers the potential of introduction of *Lm* into the RTE processing environment from an outside source or through disturbance of a harborage site (e.g., the process of replacing floor drains, walls, or cooling units) as a great concern.

Control of the Environment during Construction

If possible, suspend operations during construction. Otherwise:

- Dust from construction can be difficult to detect and control. Therefore, increased monitoring of product, food-contact surfaces, and the environment is recommended during and after these disruptive events.

- Establish negative air pressure in the construction area in order to ensure that air does not flow from the construction area into the plant.

- Temporary partitions can be established to protect the undisturbed areas of the plant from construction dust and debris.

- Cover any construction debris when moving out of the construction area.

- Do not move debris through RTE processing areas or areas that directly connect to RTE processing areas, if possible.

- Schedule construction during non-processing hours.

- Conduct intensified cleaning and monitoring of food contact and environmental surfaces after construction is complete.

Control of the Environment after Construction

- Schedule removal of all construction equipment, barriers, and final debris after production hours.

- Perform a thorough clean-up and increased sanitation sampling at pre-operational inspection. Continue intensified cleaning and monitoring of food contact and environmental surfaces until food contact surfaces test negative for 3 consecutive days.

V. Intensified Cleaning and Sanitation Following a Positive *Listeria* Sample

The following are actions that can be taken during intensified cleaning. Not all steps may be necessary to address contamination. Actions should be escalated to address consecutive positives.

If positives occur, consider:

- Thoroughly cleaning and scrubbing sites where positives were found.
- Identifying all possible harborage sites and cross contamination pathways. Clean and sanitize harborage points and address cross contamination.
- Removing equipment parts and soaking overnight.
- Increasing the frequency of all less than daily sanitation procedures (e.g., walls and ceilings).
- Scrubbing surfaces where product residue accumulates. Pay special attention to gaps, cracks, rough welds, and crevices in equipment.

If positives continue to occur, consider:

- Disassembling equipment and soaking of parts in quaternary ammonia overnight.
- After cleaning and sanitizing of larger pieces of equipment, applying steam heat via an oven at 160^0F and holding for 20-30 minutes.
- Fogging the room with a sanitizer solution.
- Replacing rusty, pitted, peeling tools or parts of equipment with new, smooth-surfaced ones. These rusty, pitted tools and equipment parts serve as ideal harborage places for *Lm* to grow and multiply.

If positives still continue to occur, consider:

- Identifying harborage points in equipment, such as spiral freezers and slicers, and repairing or replacing.
- Thoroughly cleaning all areas of the establishment, including raw and non post-lethality exposed areas, to address possible harborage sites leading to contamination of RTE areas.
- Repairing or replacing leaky roofs, broken and cracked equipment, floors, overhead pipes, and cooling units, fans, doors, and windows. Suspend operations during repairs or replacement. FSIS recommends testing the environment for *Listeria spp.* after repairs are finished.

VI. Determining the Effectiveness of the Sanitation Program

Establishments can verify the effectiveness of their sanitation program through monitoring the implementation of their pre-operational and operational procedures in their Sanitation SOP. The most basic level of daily verification occurs within the post-lethality environment by monitoring the effective implementation of cleaning/sanitizing of FCSs and observing whether operational sanitation procedures are implemented to prevent cross-contamination (9 CFR 416.13(c)). Maintaining daily records to document the implementation and monitoring of the Sanitation SOP procedures targeted to the RTE environment is also a regulatory requirement to track the effectiveness of the sanitation program (9 CFR 416.16(a)). In addition, observation of employee hygiene practices within the RTE area is required to verify compliance with the Sanitation Performance Standard and prevent cross-contamination (9 CFR 416.5(c)). There are also requirements in the *Listeria* Rule for sampling for *Lm* or an indicator organism to verify sanitation. These are discussed in the main body of the *Listeria* Guideline.

It is also important that establishments take steps to prevent future contamination events. This can include reassessing and modifying the Sanitation SOP for specific pieces of equipment or areas of the establishment, increasing cleaning and sanitation frequency, and repairing or replacing equipment or areas of the establishment that may represent harborage sites for *Lm*.

Non-regulatory methods to verify the effectiveness of the Sanitation SOP include the use of total plate counts and ATP bioluminescence, as well as organoleptic inspection**. It is important to note that these methods can not be used to replace testing performed for *Lm* or an indicator organism to meet the requirements of the *Listeria* Rule.**

Total Plate Counts (TPC)

Visual verification combined with Total Plate Counts (TPCs) can determine both observable contamination and the level of bacterial contamination. Since TPC results are available in about 24 hours, and cannot be obtained at the time of inspection, their value lies in the measurement of the level of contamination. The level of contamination on cleaned and sanitized equipment should be very low (e.g., less than 100 CFU/in^2). The level of contamination may assist the establishment in determining the source of *Listeria* contamination and the effectiveness of the Sanitation SOP. Establishments may be able to use the results from TPC monitoring to indicate areas where *Listeria* spp. testing should be performed.

ATP Bioluminescence Testing “Lightning”

The use of adenosine triphosphate (ATP) bioluminescence swab testing on FCSs can also be a measurement tool to verify sanitary conditions. Most food residue and all microbes are rich in ATP and detecting microorganisms through ATP bioluminescence analysis is one method to test for sanitation effectiveness. The more ATP present, the greater the amount of bioluminescent light emitted. A microprocessor transforms the data into a digital readout for the luminometer’s display and quantifies the light output into a 2 digit zone. The product manufacturer specifies the “acceptable” and “unacceptable” zone. The ATP test can detect contamination that is not observable, is a rapid test, and results are available immediately prior to the start of operations.

It is important for the establishment to verify that the cleaning and sanitizing procedures are effective. In addition, the recordkeeping should be used for data analysis and the establishment should evaluate the monitoring records for trends. 9 CFR 416.14 requires that each official

establishment routinely evaluate the effectiveness of the Sanitation SOP and the procedures therein. Therefore, trend analysis, evaluation, and appropriate revision of the Sanitation SOP, should be conducted, as necessary, to remain effective and current with respect to changes in facilities, operations, equipment, utensils, personnel, and equipment within the post-lethality environment.

Records of Sanitation Procedures

The following sanitation records are required by 9 CFR 416.16:

- Keep records of the implementation of Sanitation SOPs.
- Maintain monitoring records of Sanitation SOPs.
- Maintain records of corrective actions taken if adulterated product or a direct FCS noncompliance occurs. Ensure appropriate disposition of products, restore sanitary conditions to prevent recurrence, and record the date of the noncompliance and the initials of the plant employee conducting the corrective action.
- Records must be maintained for 6 months, and may be stored electronically.

References

De Roin, Mark, S.C. C. Foong, P. M. Dixon, J. S. Dickson. 2003. Survival and recovery of *Listeria monocytogenes* on ready-to-eat meats inoculated with a desiccated and nutritionally depleted dustlike vector. J. Food Protection. 66: (6): 962-969.

Tompkin, R.B. 2002. Control of *Listeria monocytogenes* in the Processing Environment. Journal of Food Protection. 65 :709-725.

- Assessing the effectiveness of its PLT or AMAs or AMPs to address the increased likelihood of positives,
- Determining whether *Listeria* trends exist (see Section 4.5), and
- Reassessing its **HACCP plan,**[8] to determine if the actions it is taking are effective in controlling *Listeria*.

> **NOTE:** The finding of three consecutive positive samples from the same sampling site indicates a serious contamination issue, and increases the risk that product could be contaminated with *Lm.*

4.3 Hold and Test

According to the *Listeria* Rule, establishments in Alt. 3 (deli and hotdog producers) are required to hold product after a 2nd consecutive positive for *Lm* or an indicator organism until the establishment corrects the problem indicated by the test result (9 CFR 430.4(b)(3)(ii)(B)). Further, in order to release product into commerce, the establishment must sample and test the lots of product using a method that will provide a level of statistical confidence that the product is not adulterated (for more information see International Commission on Microbiological Specifications for Foods (ICMSF) Sampling Plans for *Lm* below). Alternatively, the establishment may rework or condemn the product (9 CFR 430.4(b)(3)(ii)(C)). Establishments in Alt. 3 (non-deli or hotdog producers) and Alt. 2b are required to identify when they will hold and test product ((9 CFR 430.4(b)(2)(iii)(B) and (3)(i)(B)) and FSIS recommends that they do so after the **3rd positive** (see Table 4.1). It is also recommended that establishments in Alt. 1 and 2a hold and test product after multiple positives for *Lm* or an indicator organism.

Establishments can include their hold and test procedures in their *Listeria* Control program. Products can be tested for either *Lm* or *Listeria* spp.; however, if a product tests positive for *Listeria* spp. an establishment may be asked to provide further evidence (such as confirmatory testing results) to demonstrate that the product is not contaminated with *Lm*.

Establishments should hold the entire product lot (and subsequent day's lots) until control is regained. For more information on defining product lots, see Section 3.5. Control is considered regained after 3 consecutive days of negative FCS results are obtained, and all other NFCS and product samples are negative. **If product tests positive for *Lm* during hold and test (see Appendix 4.2), then the product lot represented by the sample is considered adulterated.**

> **NOTE:** Control is regained after **3 consecutive days of negative** FCS results are obtained, demonstrating that corrective actions are sufficient to address the contamination issue.

A hold and test scenario is provided in Appendix 4.2 that provides a day-by-day description of hold and test procedures. Establishments should also describe product disposition in response to positives (procedures for reworked or condemning the product).

Hold and test can only be used as a means to release product in situations where an FCS tests positive for *Listeria* spp. If FCSs or product tests positive for *Lm* the product is considered adulterated. In that case, holding and testing product would not be an appropriate

[8] Reassessment of the SSOP or HACCP plan is required in response to an FSIS *Lm* positive according to 9 CFR 417.3(b) and 416.15 (b) (see the Q&As in Appendix 3.1 for more information).

consider possible hazards from ingredients (such as spices) added after the lethality treatment. In other cases, the establishment did not have supporting documentation on file, such as letters of guarantee or certificates of analysis (COA) demonstrating that the ingredients it added to product were safe and would not cause the product to become adulterated. Information on ensuring the safety of ingredients in RTE product can be found in the FSIS RTE *Salmonella* Guideline, available at: http://www.fsis.usda.gov/PDF/Salmonella_Comp_Guide_042211.pdf. Information on avoiding sources of environmental contamination can be found in the *Listeria* Guideline (see Appendix 2.2).

By reviewing the examples provided above and addressing deficiencies in their food-safety programs, establishments can help ensure that they meet the requirements of the *Listeria* Rule. In addition, by reviewing their programs to ensure that possible weaknesses are addressed, establishments can produce safe products and help protect public health.