Executive Summary

Project title: Cost Effective Treatments to Minimize *Listeria monocytogenes* Cross Contamination of Ready-To-Eat Meats by the In-Store Deli Meat Slicer

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Objectives:

1. Develop a visual verification system to insure that food contact surfaces are "clean to sight and touch."
2. Improve cleaning and sanitation methods to insure the effectiveness in removing *Listeria* and *Listeria* biofilms.
3. Assess the effectiveness of "hot boxes" to sanitize clean slicers overnight.
4. Draft "Best Practices" and test under commercial conditions for cleaning and giving deli meat slicers a lethal heating step.

Conclusions:
Two approved red food dyes, FD&C No. 3 and No. 40 vividly stain the protein and fat in bologna and turkey luncheon meats. Use of a 1:1,000 dilution of these inexpensive dyes should improve the ability of deli managers and deli personnel to quickly determine if there are areas on the slicer or environment with gross contamination and if additional cleaning is required before sanitizing the slicer or beginning operations. In a test of sanitizers against *Listeria* biofilms on aluminum or stainless steel components, the best results were obtained with J512, but there was still only about a reduction log 1.5 log CFU per coupon (or less than 0.5 log/cm^2). Barrier II also reduced Lm on the stainless by about 1.0 log CFU/ coupon, but reduced Lm on the aluminum coupon by almost 2.0 log CFU/coupon. Holding deli slicer components in dry oven conditions at 66, 77 or 82 °C, for extended times up to 15 h was not effective for eliminating *Listeria* on the slicer component surfaces. However, heating the components in moist oven conditions caused the desired 5 log reduction of *Listeria* within 3 h at 82 °C.

Deliverables: Technical Abstract:

Ready To Eat (RTE) luncheon meats sliced in a retail deli may pose the greatest risk of listeriosis, a greater risk than any of the other 23 categories of RTE foods. Previous research has shown possible cross-contamination of *Listeria monocytogenes* (Lm) on RTE meats sliced on a mechanical meat and cheese slicer. Before the slicer can be sanitized it must be cleaned. The Model Food Code requires food contact surfaces at room temperature to be cleaned to "sight and touch" every 4 hours. Our cooperators, who are deli managers have expressed an interest in an inexpensive indicator that could be used to illuminate meat or cheese residues. Histological stains for proteins, such as Coomassiee Brillant Blue, were tested but proved unsuccessful. A 1:1,000 water dilution of the common red food grade dye, FD&C No. 3 and No. 40, stains meat and fat residues on the metal surfaces of the slicer. There should be minimal regulatory concerns with the use of these dyes that significantly increases the visulation of tiny bits of luncheon meat on the surfaces of the slicer.

Four commercial sanitizers were tested against established *Listeria* biofilms. Coupons (2 cm x 2.5 cm) were cut from the stainless steel cutting blade or cast aluminum blade guard or product tray of the slicer as the components reported to be the most likely contaminated. An Lm cocktail made up of strains isolated from delis (F2365, J0161, J2818, F6900) obtained from Cornell University, Ithaca, NY were inoculated on the slicer coupons and trypticase soy broth (TSB) was added daily for 4 days to build up a biofilm layer. After 1 min of contact time the sanitizers were neutralized. The best results were obtained with J512, but there was still only about a reduction of log 1.5 log CFU per coupon (or less than 0.5 log/cm^2). PanClean reduced Lm about 1.0 log CFU/ coupon on the stainless but did not reduce Lm on the aluminum coupon. Barrier II also reduced Lm on the stainless by about 1.0 log CFU/ coupon, but reduced Lm on the aluminum coupon by almost 2.0 log CFU/coupon. The SaniWipes reduced Lm less than 1.0 log CFU/coupon for both stainless and aluminum. There was less reduction of the Lm biofilm on the more porous aluminum surface than had been expected.

A 5 log kill has been determined to be sufficient for most foods that are not low acid canned foods. In these thermal destruction tests a surrogate, *Listeria innocua* (Li) was used because it is non-pathogenic and has a heat tolerance greater than Lm. Deli slicer components were marked off in 2" x 2" grids. An inoculum of 40 µL of Li was added. Once dried the components were wrapped in heavy-duty aluminum foil to prevent aerosolization and placed in identical convection oven at either 66, 77 or 82 °C with either dry or moist heat. A water saturated atmosphere of 100% RH was used for the moist conditions. Gridded areas were sampled with sterile cotton-tipped swabs at 0.5, 1, 3, 15 h of thermal treatment. Swabs were placed in 10 mL sterile PBS, serially diluted and plated onto Trypticase Soy Agar with yeast extract (TSAYE). Plates were incubated at 37 °C for 24 h. Three replicates per each time/temperature combination thermal conditions were monitored by iron constantan thermocouples and a temperature-relative humidity by using a data logger.

Analysis indicated that holding deli slicer components in **dry oven conditions** at 66, 77 or 82 °C, for extended times up to 15 h (overnight) was not effective for eliminating Li on the slicer component surfaces. However, heating the components in **moist oven conditions caused a 5 log reduction of Li within 3 h at 82 °C**. Although high humidity/high temperature conditions were

effective, this treatment would not be feasible to use on the assembled deli slicer because of potential damage to the electrical components. Continuing research involves using various sanitizers alone and in combination with moist heat to further reduce potential Lm contamination of disassembled stainless steel and aluminum deli components.

I. Goals and Objectives

Overall Project Goals: Significant advances have been made by the meat and poultry industries to minimize contamination of ready to eat (RTE) sliced deli meats using improved sanitation and antimicrobials that suppress the outgrowth of low levels of *Listeria monocytogenes* (Lm). The next step in minimizing the risk of Lm from RTE deli foods is to direct research focused on finding more effective cleaning and sanitizing methods for the deli slicer to further reduce the risk of listeriosis. At the completion of this research, meat companies and their customers who operate delis will have additional Best Practices based on new data that demonstrates a significant reduction in Lm on the deli meat slicer. This research can reduce the cross-contamination of Lm on RTE luncheon meats and help meet consumers' desires for the convenience of RTE foods and still feel that RTE deli meats are safe for their family.

Supporting Objectives:

a) Develop a visual verification system to insure that food contact surfaces are "clean to sight and touch". It is absolutely essential that deli slicer surfaces are clean before proceeding to sanitizing the slicer.
b) Measure the effectiveness of current deli operators' recommended cleaning and sanitation practices in removing *Listeria* and *Listeria* biofilms to determine the cost effectiveness and time efficiency of improved cleaning methods that could permit the use of meat slicers for longer time intervals beyond the recommended 4 hours.
c) Assess the effectiveness of existing deli dry and moist heat ovens to sanitize clean slicers overnight for complete destruction of *Listeria* in biofilms on their food contact surfaces.
d) In cooperation with commercial deli managers, draft and test under commercial conditions "Best Practices" for cleaning and giving deli meat slicers a lethal heating step.

II. Materials and Methods

Dyes: Food grade and histochemical dyes for proteins were evaluated including Coomassie Brilliant Blue, FD&C No. 3, FD&C No. 40.

Sanitizers: Barrier II, San Wipes, J512, PanClean

Coupons: Food contact surfaces from a donated deli slicer were cut into coupons measuring 2 cm^2. Stainless steel components were cut into 2 x 2.5 cm coupons using a Flow Waterjet Cutting System (Flow International Corporation, Kent, WA) at C. Mayo Sheet Metal. This cutting system was used to prevent heat induced stress that could cause a change in the physical properties of the metal slicer components. The softer cast aluminum coupons were cut using a Milwaukee Heavy-Duty metal cold-cutting saw (Brookfield, WI) and a Wellsaw metal-cutting bandsaw (Wells Manufacturing Corporation, Three Rivers, MI). Coupons were washed with

Micro-90 detergent solution (International Products Corporation, Burlington, NJ) prepared as recommended, and scrubbed with 3M Scotch Brite® pads. Coupons were rinsed three times with deionized water, and autoclaved at 121 °C for 15 min.

Bacterial strains: *Listeria innocua* (Li) strain M1 (Fairchild and Foegeding, 1993) was obtained from the culture collection of the Food Safety Laboratory of the University of Arkansas.

Listeria monocytogenes strains Lm 4b (Cornell – FSL R2-574 - CDC associated with outbreak 1985, isolated from cheese, CA); Lm ½ a (Cornell – FSL F6-154, isolated from turkey associated with outbreak 2000; Lm ½ a (Cornell – FSL R2-499, isolated from human illness in outbreak 2000; Li (Cornell – FSL C2-008); Lm ½ a (Cornell – FSL R2-564, isolated from single case of human listeriosis 1989) were obtained from colleagues at Cornell University, Ithaca, NY.

Stock cultures were kept frozen at -80^{o} C. To reactivate cultures one loop of frozen stock was inoculated into 10 ml sterile tryptic soy broth plus 0.6% yeast extract (TSBYE; BD, Franklin Lakes, NJ) and incubated at 37 °C for 24 h.

Biofilm development: The protocol of Pan and others (2006) was followed to prepare coupons to grow measurable *Listeria* biofilms. The sterile coupons were aseptically placed in sterile 6 well plates (Falcon, Multiwell™ 6-well, flat bottom plates, Becton-Dickinson Labware, Franklin Lakes, NJ), containing sterile 3.2 cm Whatman filter paper (Whatman International, Ltd., Maidstone, England) that was slightly moistened with sterile deionized water to aid in keeping relative humidity at 100%. A cocktail of Lm cultures was made by mixing equal volumes of each culture, and 0.1 ml of the cocktail was pipetted onto each sterile coupon and spread evenly with a disposable inoculation loop and allowed to dry at 24 °C for 3 h. Afterwards, 0.1 ml of TSBYE was added to each coupon and incubated for 14 h. To remove free cells, the coupons were washed carefully by rinsing with 1 ml of sterile potassium phosphate buffer saline (PBS – 50 mM, pH 7.0). Afterwards, 0.1 ml of sterile tryptic soy broth (TSB, BD, Franklin Lakes, NJ) was added to each coupon. The plates were incubated at 37 °C for 24 h. TSB was added daily for 4 days to build up biofilm layer (per Moltz and Martin 2005).

Sanitizer testing: Stainless steel and cast aluminum coupons with biofilms were placed in a Biosafety cabinet. Sanitizers used in retail delis were prepared per manufacturer's instruction and predetermined amounts were placed onto each biofilm. Sanitizers tested included SaniWipes, PanClean, J512 and Barrier II. Sanitizers were allowed to react with biofilm for 60 s. Immediately after this time, 1 ml of D/E neutralizing broth was added to each biofilm coupon to halt the reaction. After 1 min, excess D/E broth was poured off the surface of the coupon. Coupons were swabbed with calcium alginate tipped swabs. Swabs were placed in a culture tube containing 9 ml of sterile PBS at pH 7.0. Solution was vortexed, serially diluted and plated onto Modified Oxford Agar (MOX; Becton Dickinson and Co., Sparks, MD) agar . Plates were incubated at 37 °C for 48 h and enumerated.

Thermal inactivation testing: Deli slicer components were marked off in 4 cm^2 grids and 40 µL of Li inoculum was pipetted into the grids and allowed to dry (Figure 1). Slicer components were wrapped in heavy-duty aluminum foil to prevent aerosolization and placed in identical

convection ovens (Power-O-Matic 60, Blue M. Electric Company, Blue Island, IL) at either 66, 77 or 82 °C with either dry or moist heat. A water saturated atmosphere at 100% RH was used for the moist conditions. Gridded areas were sampled with sterile cotton-tipped swabs at 0.5, 1, 3, 15 h of thermal treatment. Swabs were placed in 10 mL sterile PBS, serially diluted and plated onto Trypticase Soy Agar with yeast extract plates (TSAYE; Becton Dickinson and Co., Sparks, MD). Plates were incubated at 37 °C for 24 h. Three replicates per each time/temperature combination of thermal conditions were monitored by iron constantan thermocouples and a temperature-relative humidity data logger.

III. Results

Indication of cleanliness:

Coomassie Brilliant Blue was not sufficient in obtaining a striking visual observation (Figure 2). Two approved red food dyes, FD&C No. 3 and No. 40 (Figure 3) vividly stain the protein and fat in bologna and turkey luncheon meats. These two dyes are also capable of transferring to the meat slicer (Figure 4). These or similar dyes can be used after washing and cleaning the deli slicer to enable deli personnel to quickly determine if there are areas of gross contamination and if additional cleaning is required before sanitizing the slicer or beginning operations.

Cleaning:

Results from testing the currently used cleaners and sanitizers against an established *Listeria* biofilm are shown in Figure 5. Four commercial sanitizers were tested against an established *Listeria* biofilm on 2 cm^2 coupons cut from the slicer. In general it appears that the stainless components may be slightly easier to sanitize than the aluminum pieces. The best results were obtained with J512, but there was still only about a reduction log 1.5 log CFU per coupon (or less than 0.5 log/cm^2). PanClean reduced Lm about 1.0 log CFU/ coupon on the stainless but did not reduce Lm on the aluminum coupon. Barrier II also reduced Lm on the stainless by about 1.0 log CFU/ coupon, but reduced Lm on the aluminum coupon by almost 2.0 log CFU/coupon. The SaniWipes reduced Lm less than 1.0 log CFU/coupon for both stainless and aluminum. Our industrial partner in this testing relies on the use of SaniWipes to wipe the slicer between uses; these results call into question whether this is an adequate control measure in the working deli.

Thermal inactivation:

Holding deli slicer components in **dry oven conditions** at 66, 77 or 82 °C, for extended times up to 15 h was not effective for eliminating Li on the slicer component surfaces (Figure 6). However, heating the components in **moist oven conditions caused a 5 log reduction of Li within 3 h at 82 °C** (Figure 7). Although high humidity/high temperature conditions were effective, this treatment would not be feasible to use on the completely assembled deli slicer because of potential damage to the electrical wiring. It would be a viable option for disassembled food contact surfaces of the slicer.

Best practices:

As a portion of this objective we have produced and laminated signs for procedures for cleaning the slicer, and translated these into Spanish (Figure 8). While working with our industrial partner

we became aware that even though we have enough information at this time to produce a "Best Practices" document in regards to the deli slicer, this will not be successful if we cannot motivate employees to follow the regimen we recommend. Further study is needed on how best to motivate workers to impliment our Best Practices.

Recommendations for future research:
We will be pursuing 4 additional objectives for the next phase of this research.

Objective a. We will examine the efficacy of a more sensitive Fluorescein dye that may need to be removed, but could verify very low levels of contamination on the slicer.

Objective b. We are expanding our Phase I results to test seven additional cleaning and sanitizing compounds, representing the range and classes of cleaners used in the retail environment. These will be tested against 20 persistent and 20 non-persistent strains of Lm recovered from actual operating deli environment.

Objective c. Response surface methodology will be used in combination with a statistical design to optimize the treatment combinations of: cleaner, sanitizer, moist heat time and temperature to achieve a 5 log reduction of Lm on deli slicers. We hypothesize that moist heat and chemical sanitizer combinations may significantly decrease the temperature requirements. We will evaluate novel combinations of sanitizers that do not require rinsing in combination with moist heat to achieve a 5 log kill. However, we will also determine D and z values for moist heat alone that may be required to kill Lm in harborages on the slicer not contacted by sanitizing agents. It is well established that most microorganisms are much more resistant to sanitizers and heat in their stationary rather than their log phase of growth. We will test these deli isolates in two growth phases and in a real world situation in which the inoculum is protected by a luncheon meat emulsion and dried on the slicer's surface.

Objective d. Working with our industrial partner we will continue to develop and test the Best Practices for the deli slicers. We will also work with employees in delis to determine motivation to follow these practices, and to develop training programs for deli managers and employees that will be engaging and effective.

Presentations and Publications:
Results were shared in a Powerpoint presentation to John Clark, Director of Bakery and Deli Operations, Harps Food Stores, Inc., Springdale, Arkansas on July 18, 2008.

Thermal Inactivation of *Listeria innocua* as a surrogate for *Listeria monocytogenes* on Deli Slicer Components, Elizabeth M. Martin, Sean Pendleton, Erin Shannon, Sara R. Milillo, Philip G. Crandall, Steven C. Ricke, Michael G. Johnson, Corliss A. O'Bryan, and John A. Marcy. Poster presentation at ASM annual meeting May, 2009.

A paper is in preparation for Journal of Food Protection on thermal inactivation of Li and Lm in dry and wet environments.

References:

Best M, Kennedy ME, Coates F. 1990. Efficiacy of a variety of disinfectants against Listeria spp. Appl Envir Micro 56:377-380.

Fairchild, T.M., and Foegeding, P.M.1993. A proposed nonpathogenic biological indicator for thermal inactivation of *Listeria monocytogenes*. Appl Environ Microbiol. 59: 1247-1250.

Friedly EC, Crandall PG, Ricke S, O'Bryan CA, Martin EM, Boyd LM. 2008. Identification of Listeria innocua surrogates for Listeria moncytogenes in hamburger patties. J Food Sci 73:M174-M178.

Gombas DE, Yuhuan C, Clavero RS, Scott VN. 2003. Survey of *Listeria monocytogenes* in ready-to-eat foods. J Food Prot 66:559-569.

Moltz, AG, Martin,SE. 2005. Formation of biofilms by Listeria monocytogenes under various growth conditions. J Food Prot 68:92-97.

Pan, Y, F. Breidt, Jr., and S. Kathariou. 2006. Resistance of *Listeria monocytogenes* biofilms to sanitizing agents in a simulated food processing environment. Appl Environ Microbiol 72: 7711-7717.

USDA/FSIS. 2003. Draft FSIS risk assessment for *Listeria* in ready-to-eat meat and poultry products. Available at http: //www.fsis.usda.gov/OPHS/lmrisk/DraftLm22603.pdf. Accessed Oct. 2007

Figure 1: Cast aluminum component with marked gridded area after inoculation of 40 ul *L. innocua*.

Figure 2. Coomassie Brilliant Blue used on bologna, subsequently sliced by knife.

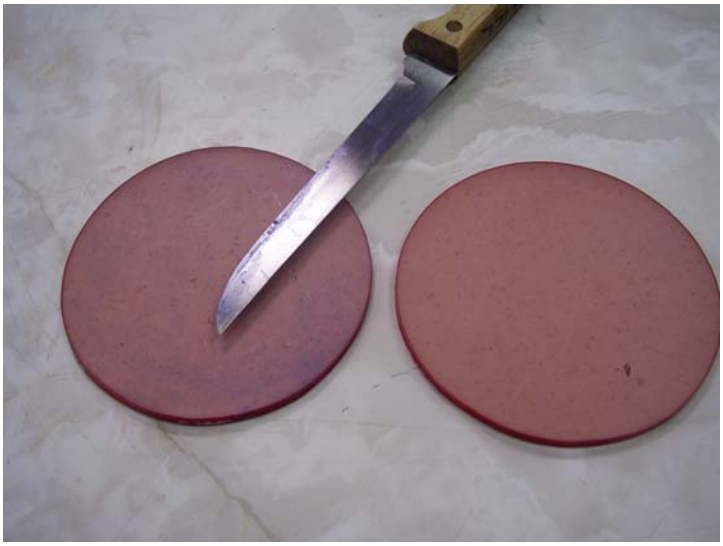

Figure 3. Food grade red dye, FD&C No. 3 and No. 40, on sliced bologna and turkey breast deli meats. (Controls, without dye, on the left)

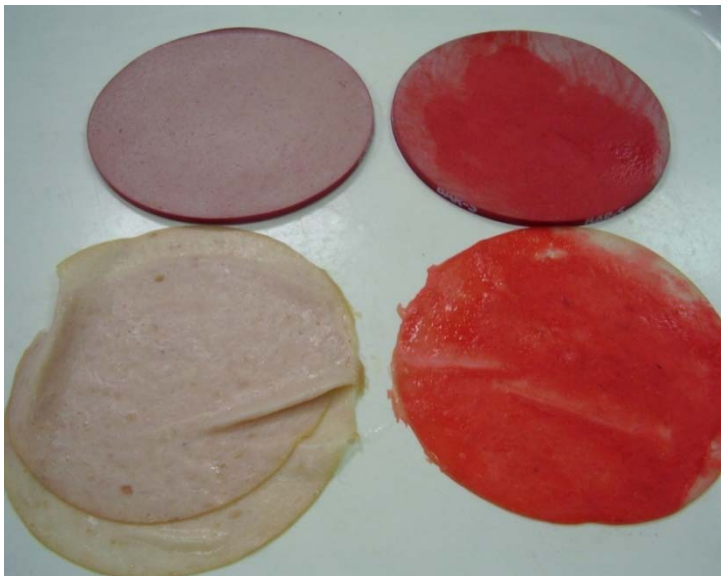

Figure 4: Deli slicer meat holder stained with food grade red dye after contact with luncheon meats (FD&C, no. 3 and no. 40).

Figure 5: Log reduction in *Listeria monocytogenes* by sanitizer

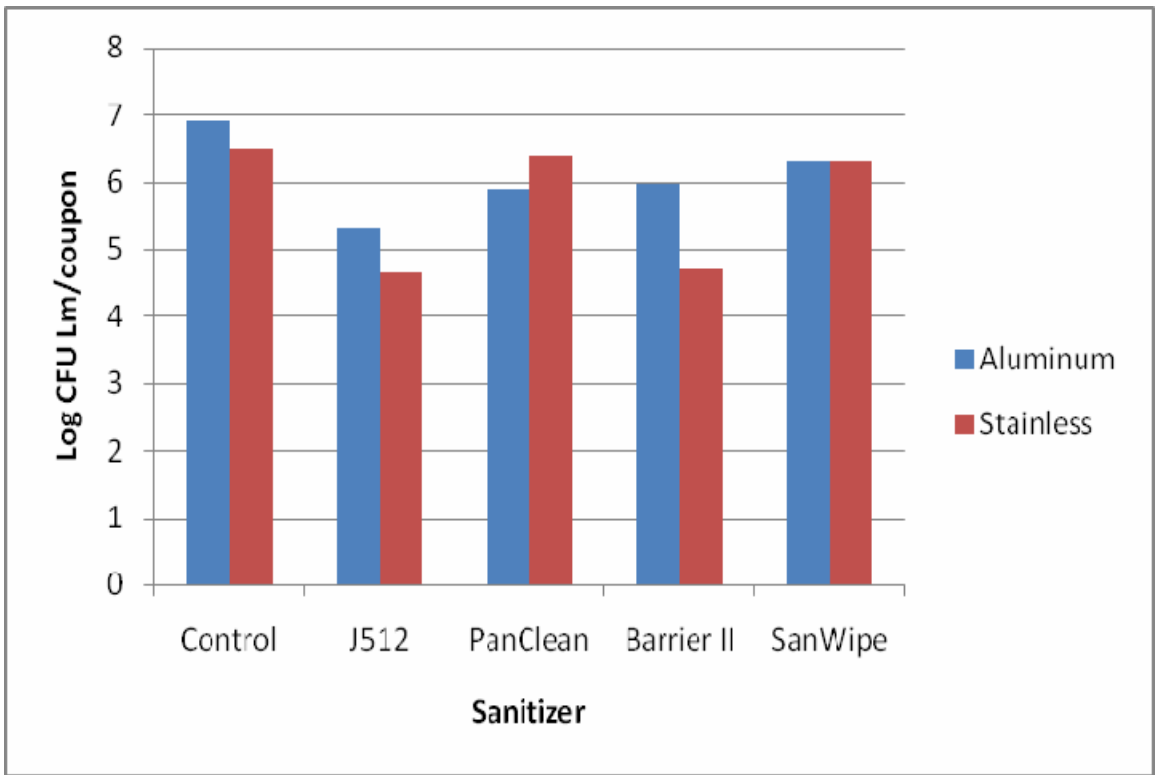

Figure 6. Analysis of thermal inactivation of *Listeria innocua* surface-contaminated onto deli components and heated at 66, 77 or 82 ^{o}C. Log CFU/cm^2 reflects the survival of *L. innocua* post heat treatments.

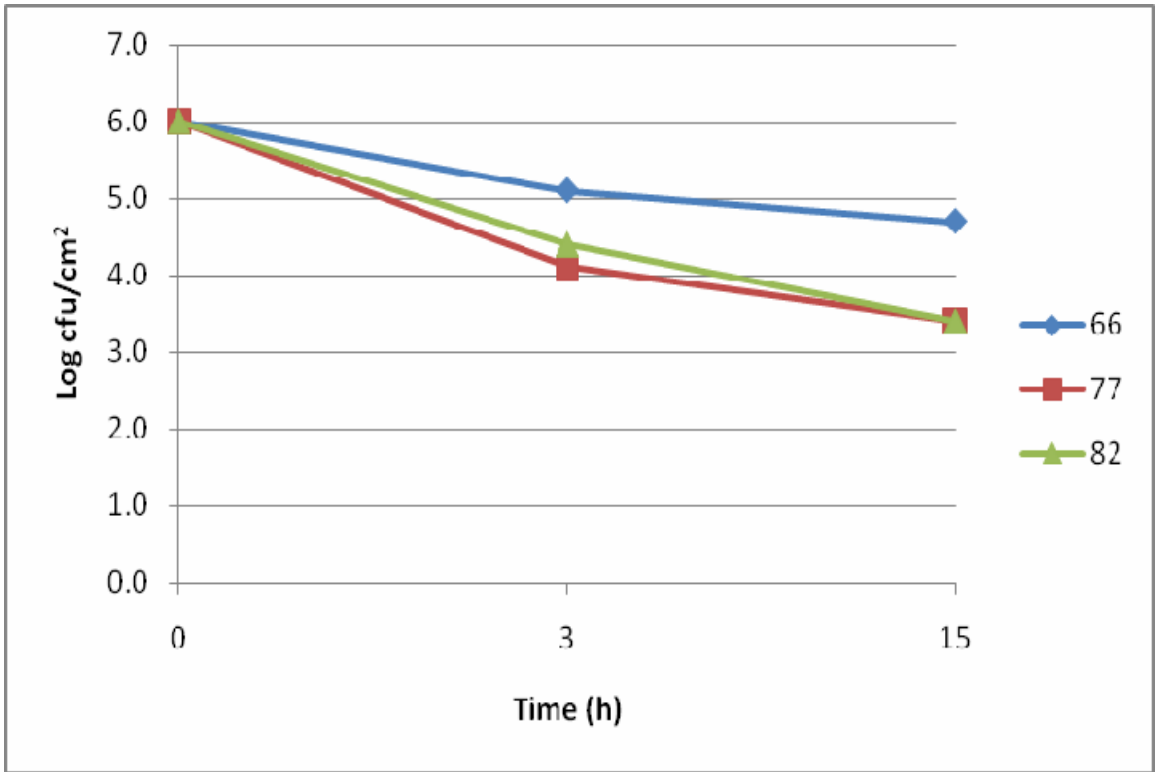

Figure 7: Analysis of thermal inactivation of *Listeria innocua* surface-contaminated onto deli components and heated at 71 or 81 ^{o}C. Log CFU/cm^2 reflects the survival of *L. innocua* post heat treatments.

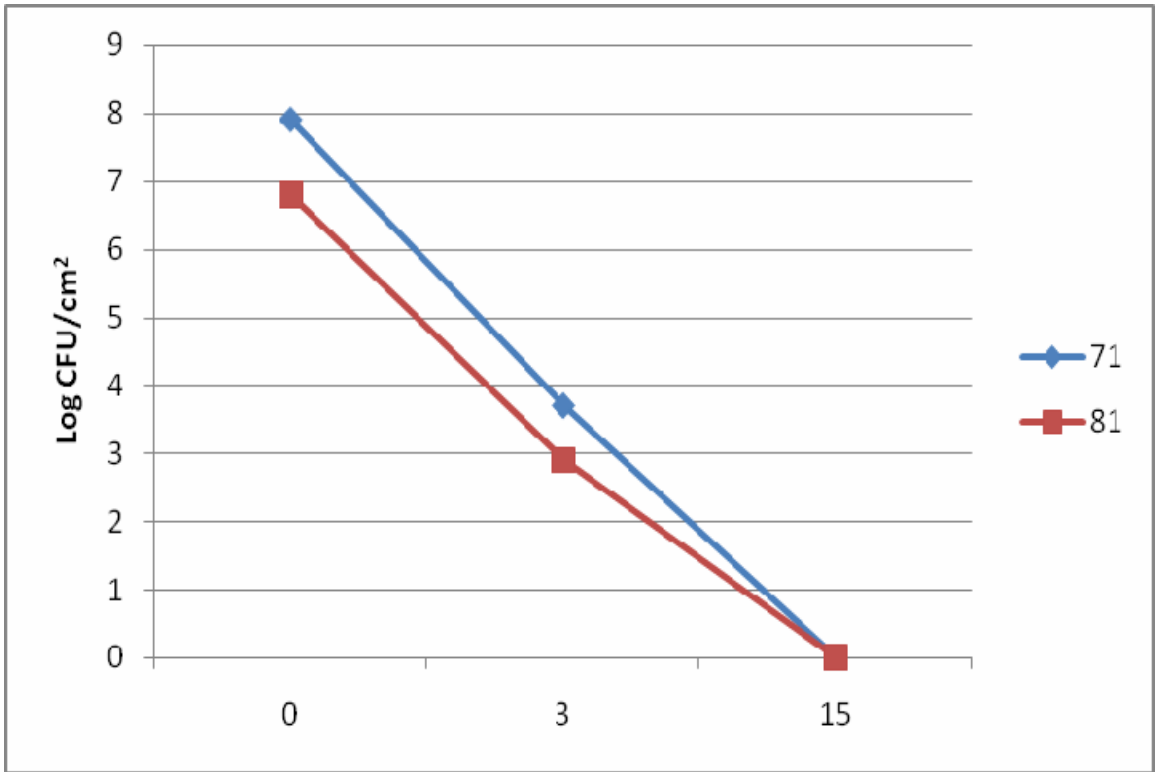

Figure 8: "Best Practices"

As a portion of this objective we have produced and laminated signs for procedures for cleaning the slicer, and translated these in

(computer designed by David G. Martin, character animator)