

Research Project Status Report

Project: 08-211

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Organization: University of Arkansas

Title: Cost Effective Treatments to Minimize In-Store Deli Meat Slicer Cross Contamination of Ready-To-Eat Meats by *Listeria monocytogenes*, Phase II

Cost: \$88,000

Timeline: Two years

End Date: March 2011

Description: The objectives for this research follow:

Objective a. During Phase I of this project, it was determined that an inexpensive food grade dye could verify contamination on the deli slicer and its components. The objective is to examine a more sensitive Fluorescein dye that may need to be removed, but could verify very low levels of contamination. Confirmation will be made by commercial ATP swab.

Objective b. Phase I results are being expanded to test seven currently used cleaning and sanitizing compounds representing the range and classes of cleaners used in the retail environment. These cleaning compounds will be tested against 20 persistent and 20 non-persistent strains of *Listeria monocytogenes* (*Lm*) recovered from the actual operating deli environment. These retail cleaning and sanitizing compounds will be tested for their activity against *Lm* in a BS level II lab.

Objective c. Response surface methodology along 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 will be used. It is hypothesized that moist heat and chemical sanitizer combinations may significantly decrease the temperature requirements. Novel combinations of sanitizers that do not require rinsing will be evaluated in combination with moist heat to achieve a 5 log kill. However, the D- and z-values for moist heat alone required to kill *Lm* in harborages on the slicer that do not come in contact with sanitizing agents will be determined. 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. These deli isolates will be tested 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. It is assumed that no matter how detailed initial employee training methods, there is still a need for constant visual reminders for deli employees to re-enforce the vitally important proper cleaning and sanitizing procedures. Ultimately, it is crucial that consumers are sold RTE deli meats that have been sliced with a sanitary in-store deli slicer. A recently created pictorial poster describing proper cleaning and sanitizing of the deli slicer and its components will be reviewed by deli managers, tested in-store and, if needed, updated.

Status:

Objective a. During Phase I of this project, it was determined that an inexpensive food grade dye could verify contamination on the deli slicer and its components. A food-safe fluorescent dye (Glo Germ) was chosen for the study.

Researchers are cooperating with Elliot Ryser, MSU, who has already demonstrated that with a fluorescent indicator (FI) it was possible to identifying 5 product contact surfaces on the deli slicer that were cross-contaminated following the slicing of Glo Germ contaminated cooked, RTE turkey chubs (Vorst et al, 2004). Researchers are cooperating in the writing of the NIFSI grant and propose to compare four FI that are all commercially available are GermaGlo, GlitterBug®, Glo Germ and Clue Spray.

Objective b. To identify the optimum sanitizer, previous studies evaluated strains of *Lm* that were implicated in listeriosis outbreaks and *Listeria innocua* (*Li*) M1, known for its persistence, and Barrier II was identified as an optimum sanitizer. In the previous report, it was determined that Barrier II, which is used now by the deli employees in the cooperative retail deli stores, is the optimum sanitizer that has been tested thus far in the lab. These results are being extended to optimum ways to sanitize/disinfect the deli slicer surfaces and other deli environment surfaces.

Research is now focused on various wiping and/or sanitizing cloths that are used to wipe down the deli slicer and surfaces in the deli. The deli slicer components were inoculated with the *Lm* and *Li* strains and test the efficiency of the cloths to reduce/eliminate these contaminants. Various cloths used to wipe down these surfaces were tested:

1. Sani-Wipe (PDI, the Healthcare Division of Nice-Pak Products, Inc. Two Nice-Pak Park, Orangeburg, NY). Sani-Wipes' active ingredients are n-Alkyl, dimethyl benzyl ammonium chloride - 0.0175%, isopropyl alcohol - 5.4800%;
2. 100% cotton terry towels (CINTAS supplies these towels to our cooperative stores. They were purchased from American Dawn, Inc., Compton, CA);
3. Textronic microfiber cloth (VERMOP Deutschland GmbH, Frankfurt, Germany);
4. Softronic microfiber cloth (VERMOP). Cloths at been placed vacuum sealed and sterilized by autoclaving.

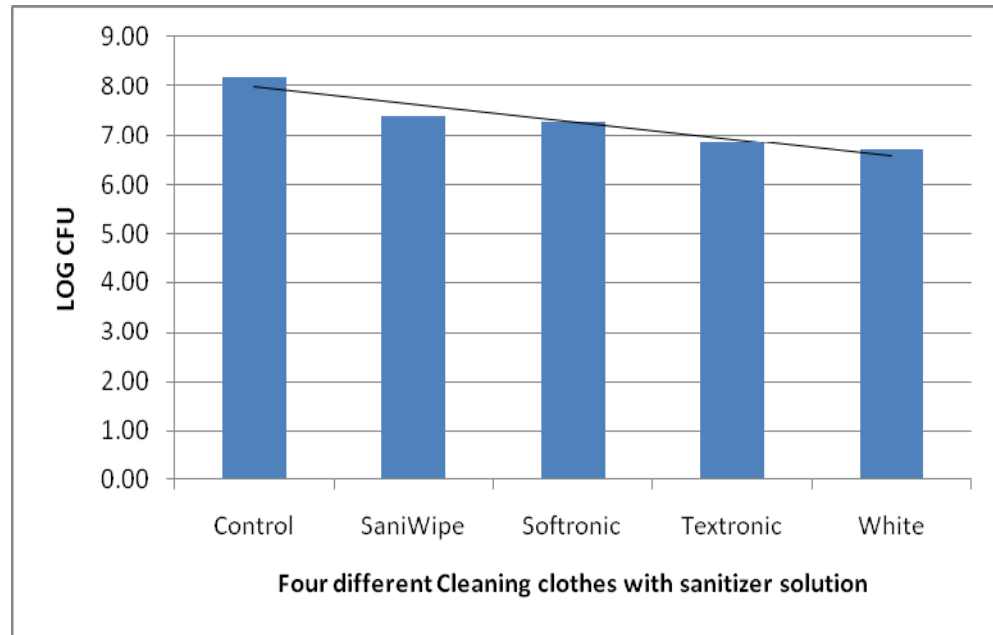
Listeria cultures for *Lm* test cocktail included: *Lm* strains from Cornell University - 27- 4b, 98 - ½ c, 187 - 4b, 189 ½ a, 190 - ½ a, 191 - ½ a, and *Li* strain 169 - known as M1. Cultures were grown in Tryptic soy broth, containing 0.6% yeast extract, overnight at 37 °C. Cocktail was comprised of equal aliquots of these

cultures. Inocula (100 uL) was pipetted onto 2X2 cm deli component aluminum coupons, under a BL2 Biosafety cabinet. Inocula were allowed to dry for 2 h under the cabinet.

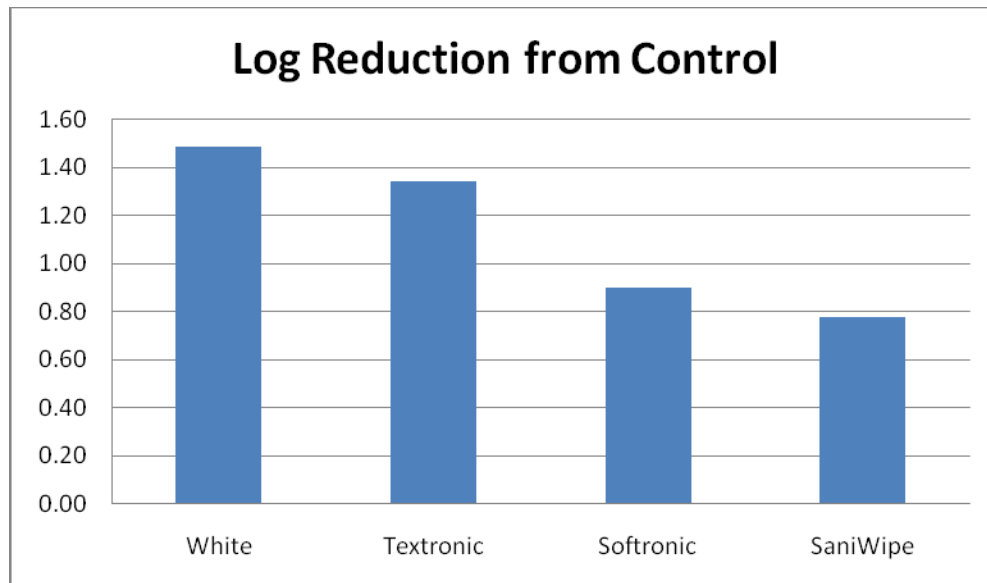
Wetness of Sani-Wipes was determined by dry weight and that amount of residual moisture (170-180%) was adjusted for each cloth type by pipetting sterile distilled water onto the cloths.

Prepared cloths were wiped across the inoculated coupons, 3X vertical, 3X horizontal. The wiped stainless-steel coupons were placed in 50 mL conical centrifuge tubes containing 10 mL of sterile PBS, pH 7.2. The tubes were sonicated for 1 min. Serial dilutions were plated onto Modified Oxford media agar (MOX) and incubated at 37°C for 48 h.

Results indicated that the greatest log reduction from the control was the white terry cloths and the lowest log reduction was from the SaniWipe. The next step should focus on the effects of using these towels with Barrrier II sanitizer where we would anticipate a greater than a 1.5 log reduction.



Control	SaniWipe	Softronic	Textronic	White
8.18	7.40	7.28	6.84	6.69



Log reduction from control - higher is better			
White	Textronic	Softronic	SaniWipe
1.49	1.34	0.90	0.78

Objective c. Previous studies showed that a combination of cleaning, sanitizing and moist heat can significantly reduce the *Lm/Li* contamination by greater than 5 logs. These techniques are being extended on a prototype using UV light as an antibacterial source. It is hypothesized that this combination will aid in the elimination of *Lm/Li*. The *Lm/Li* strains will be inoculated on deli slicer component coupons, allow a biofilm to form, then the coupons will be cleaned, sanitized and wiped. Afterwards, the coupons will be attached to the blade from which they were cut, and the UV light source will be applied.

Earlier studies determined the use of cleansing, sanitizing and moist heat had helped in the reduction/elimination of *Lm* on the surfaces of the deli slicer components. From that study, researchers are continuing to determine an interim step in the process that would also aid in the control of *Lm* on these components.

It is known that UV light has been effective in eliminating bacterial pathogens, as used as a sanitation device in Biosafety cabinets, suggesting a possible intervention for the deli environment. Because of the concern for the safety of the deli employees, we have manufactured a prototype that would protect the employees from the destructive UV rays.

For this experiment, the same strains of *Lm* and *Li* were used as inocula and were grown as stated.

Stainless steel coupons from the deli slicer blade component were contaminated by pipetting 100 uL of inocula onto each coupon. The inocula on the coupons were allowed to dry for 2 h. The coupons were placed into sterile 6 well plates. and incubated at 24°C for 3 h. The coupons were held by sterile forceps and washed 3X with 100 uL sterile PBS to remove unattached bacterial cells. Coupons were placed in new 6 well plates and 10 uL of tryptic soy broth with 0.6% yeast extract was added to each well. The plates were incubated at 24°C for

48 h. This last process was repeated to produce biofilm. The coupons were attached to the deli slicer blade in similar positions where they were removed by small magnets. The cover was placed over the UV light apparatus and the UV light remained on for 4 h. The light was then turned off, coupons were removed and placed into 50 mL conical tubes containing 10 mL of PBS. The tubes were vortexed and serial dilutions were plated on MOX agar. Plates were incubated at 37 °C for 48 h. Inocula levels were at 8.5 logs. Average control coupons (coupons with biofilm without UV treatment were at 5.7 logs). Average log reduction from control was approximately 4 logs.

Preliminary results.

Attached 4 coupons with biofilm (using a small magnet) to the UV deli slicer prototype								
Dilution	Coupon				Log CFU/area			
	1	2	3	4	1	2	3	4
-4	0	0	0	0				
-4	0	0	0	0				
-3	0	0	0	0				
-3	0	0	0	0				
-1	30	0	30	200	1.48	0	1.48	2.30

Prototype of UV apparatus. Front has plexiglass that would protect employees from UV damage. Apparatus can easily be clamped onto the sides of the deli slicer components.



Objective d. A pictorial poster, originally in English, was translated to Spanish. This poster will be presented to deli managers for evaluation.