Efficacy of a UV-C Device to Control Contamination by *Listeria monocytogenes* on Environmental Surfaces

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Objective: Determine the efficacy of a UV-C Device to Control Contamination by *Listeria monocytogenes* on environmental surfaces with different exposure times.

Introduction: Food processing companies invest significant resources into controlling environmental contamination. With manufacturers of ready-to-eat (RTE) foods, an important concern is the control of *Listeria monocytogenes*. Both the US Food and Drug Administration and the USDA Food Safety and Inspection Service have a "zero tolerance" for *L. monocytogenes* in RTE foods. That is, if a regulatory sample tests positive for the bacterium, the product must to be recalled.

L. monocytogenes is well documented as an environmental bacterium. It is easily destroyed by common sanitation practices and chemicals, but is capable of growing and surviving in environments which can be difficult to clean. Because of this, the food industry is always looking at better ways of controlling environmental contamination in processing areas, especially in those which handle RTE foods. Although there is no regulatory standard for surviving microbial populations on food processing equipment after sanitation, the food processing industry often uses a standard of either 100 or 60 ($\log_{10}$ 2.0 or $\log_{10}$ 1.78) colony forming units/cm^2. The disinfecting properties of Ultraviolet light are well known, but the practical application of the technology in food processing environments has often been difficult. This study evaluates the efficacy of a small UV instrument to reduce environmental contamination in a meat processing environment.

Methods:

Cultures and Inoculation: Fresh ground beef was mixed with sterile saline in a 1:2 ratio (1 part meat, 2 parts BPW). The mixture was homogenized by stomaching for 2 minutes, and the resulting solution centrifuged at 500 G x 5 minutes. The supernatant was used as an inoculation fluid.

Cultures of *Listeria monocytogenes* (strains H7969, H7764, H7769, H7762, and Scott A) were grown individually in trypticase soy broth with 1% yeast extract (TSB-YE)

for 18 hours at 37ºC. On milliliter each of the overnight cultures was added to a single 250 ml volume, which was incubated at 37ºC for 18 hours. The cells were harvested by centrifugation (10,000 G x 10 minutes), and the supernatant decanted off. The supernatant from the ground beef/saline preparation was added to the bacterial pellet, and the cells were resuspended by vortexing. The resulting solution contained naturally occurring bacteria from the ground beef, but with the predominant microbiome being the mixed culture of *L. monocytogenes* (>99.99% of the total population).

A foam paint brush was used to apply the *Listeria* cells to sections of the surfaces in the Experimental Meat Processing laboratory. The surfaces included floor tile, stainless steel, plastic and tile grout. The tile grout was purchased as a prepared premixed grout, which was placed in standard 100 x 15 mm petri dishes and allowed to harden for several days before inoculation. The inoculated surfaces were allowed to dry for at least 30 minutes at 10ºC prior to the application of the UV intervention.

UV Application: The UVC instrument was positioned at approximately 2 meters directly above the inoculated floor tile. The distance from the light source to the inoculated floor tile was 2 meters. The unit was operated following the manufacturer's instructions for 60, 120 and 240 minute exposure periods.

Sample Analysis: Prior to operating the unit, an 5 x 10 cm (50 cm^2) area of each surface was swabbed using a template. The surfaces were swabbed using a rehydrated sponge in a side to side motion and placed into a Whirl-Pak bag. When the exposure time was completed, a second 5 x 10 cm area was swabbed, separate from the original area. The samples were analyzed by pour plating on trypticase soy agar and incubating at room temperature for 72 hours, which would assure the recovery of sub-lethally injured cells.

Statistical Analysis: The experiment was independently replicated twice with two technical replications within each independent replication. The populations were transformed to $\log_{10}$, and the $\log_{10}$ reductions were calculated by subtracting the $\log_{10}$ population after treatment from the initial $\log_{10}$ population prior to treatment (control sample). The reductions were then analyzed with Winks SDA ver. 7.0 (Texasoft, Cedar Hill, TX). The data were modeled using the analysis function of SigmaPlot ver 13 (San Jose CA).

Results and Discussion:

The recovered populations after exposure are shown in Figure 1. The $\log_{10}$ population reductions in colony forming units/cm2 are summarized in Table 1, and the original experimental data is given in Appendix 1. Both exposure time and surface type were statistically different ($P<0.001$; Appendix 2). The greatest population reductions were seen on stainless steel, while the least reductions were seen on grout. The reductions on plastic and tile were not statistically different from each other ($P >0.05$).

As expected, the longer exposure time produced the greatest reductions, although there was no statistically significant difference between 120 and 240 minutes of exposure ($P>0.05$). Increasing the exposure time increased the $\log_{10}$ reduction, although the magnitude of reduction was greatest at the 60 minute exposure time (Table 1). Increasing the exposure time from 60 to 240 minutes resulted in an increased $\log_{10}$ reduction, but of a smaller magnitude than seen between 0 and 60 minutes.

The grout surface had the smallest reduction in population, although the inoculated population was reduced by 3 $\log_{10}$ after 240 minute exposure. The grout surface was the most porous of the four surfaces evaluated, and it is not surprising that this was the most difficult surface to reduce *L. monocytogenes* on. The porous nature of grout results in a surface which is difficult to clean and sanitize by any method, as it provides a degree of physical protection for the bacteria. UV light travels in straight lines, and it is easy to imagine how a bacterial cell, in the porous grout, could be out of the direct line of UV irradiation. A 3 $\log_{10}$ reduction in population, under these conditions with a very heavy inoculum, is still an important finding. The other surfaces (tile, steel and plastic) were much smoother and do not provide the degree of harborage that the grout provides, and are therefore easier to sanitize.

The reductions are consistent with the effects of UV irradiation on a mixed microbiome (Morey et al., 2010). Many bacteria are sensitive to UV irradiation, and are destroyed rapidly. This accounts for the large increase observed after 60 minutes of exposure. However, even within relatively homogeneous populations such as the one used in this experiment, as the most sensitive bacteria are eliminated from the population, the surviving population is of course more resistant. Because the most sensitive bacteria are eliminated after a 60 minute exposure, the surviving population shows a smaller reduction between 60 and 240 minutes, as it consists of bacteria which are less sensitive to UV.

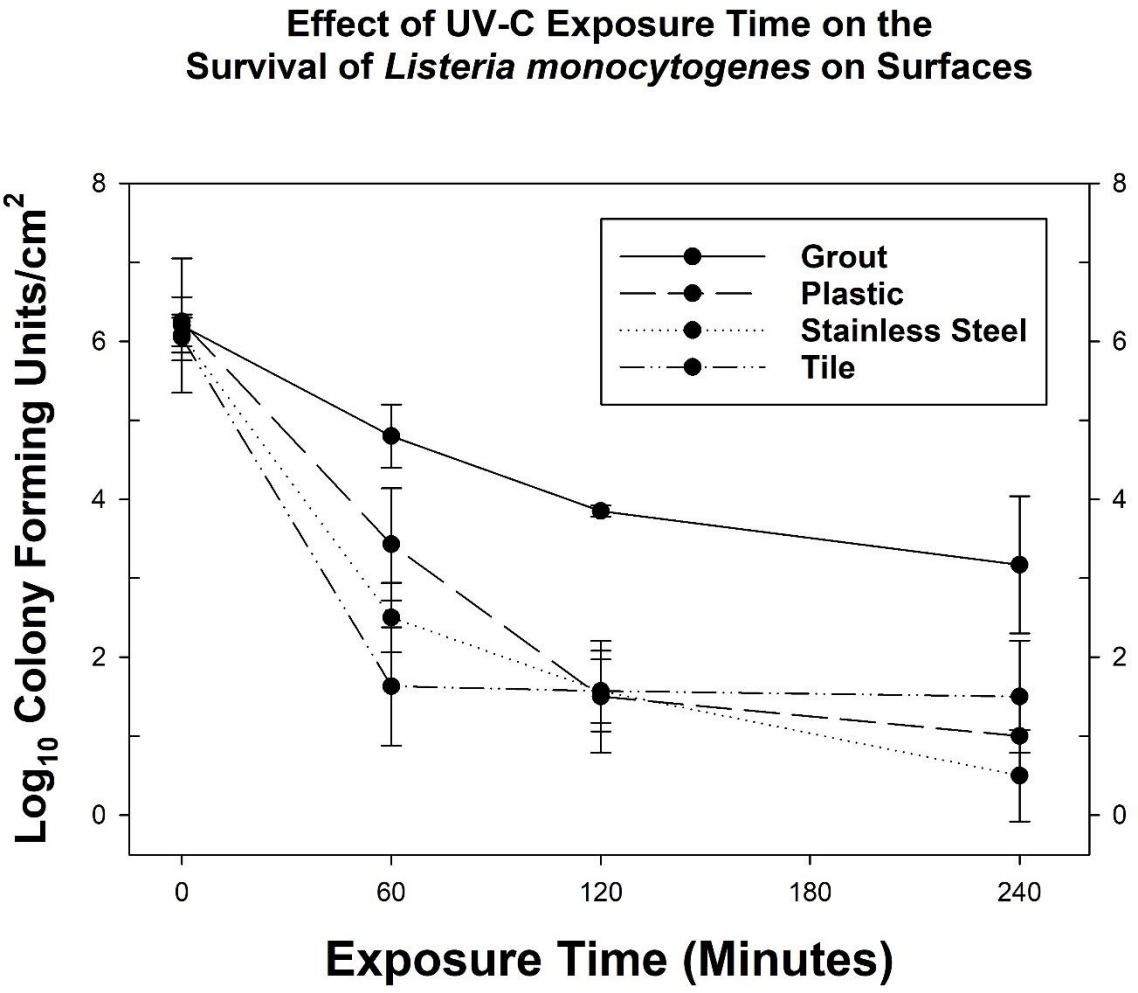
References:

Morey, A., S.R. McKee, J.S. Dickson and M. Singh. 2010. Efficacy of Ultraviolet Light Exposure Against Survival of Listeria monocytogenes on Conveyor Belts. Foodborne Pathogens and Disease. June 2010, 7(6): 737-740.

Table 1. Log_{10} reductions in the populations of *L. monocytogenes* on surfaces as affected by exposure time.

Exposure Time (minutes)	Stainless Steel	Plastic	Tile	Grout
0	0	0	0	0
60	3.58	2.82	4.42	1.4
120	4.51	4.75	4.48	2.35
240	5.58	5.25	4.55	3.03

Figure 1. Effect of Exposure time on the survival of *L. monocytogenes* on inoculated surfaces.



Appendix 1. Experimental Data.

Surface	Surface Number	Exposure Time	Rep	Population cfu/cm^2	Population log10 cfu/cm^2
Grout	1	0	2	1.4E+07	7.1
Grout	1	60	2	1.6E+05	5.2
Grout	1	120	2	6.4E+03	3.8
Grout	1	240	2	1.7E+02	2.2
Grout	1	0	3	2.6E+05	5.4
Grout	1	60	3	2.4E+04	4.4
Grout	1	240	3	8.3E+03	3.9
Grout	1	0	4	1.3E+06	6.1
Grout	1	60	4	5.7E+04	4.8
Grout	1	120	4	7.8E+03	3.9
Grout	1	240	4	2.2E+03	3.4
Plastic	2	0	1	4.5E+06	6.7
Plastic	2	60	1	2.1E+04	4.3
Plastic	2	0	2	1.4E+06	6.2
Plastic	2	60	2	2.2E+05	5.4
Plastic	2	0	3	1.3E+06	6.1
Plastic	2	60	3	9.9E+01	2.0
Plastic	2	120	3	9.9E+01	2.0
Plastic	2	240	3	9.9E+00	1.0
Plastic	2	0	4	9.1E+05	6.0
Plastic	2	60	4	9.9E+01	2.0
Plastic	2	120	4	9.9E+00	1.0
Plastic	2	240	4	9.9E+00	1.0
Stainless steel	3	0	1	1.9E+06	6.3
Stainless steel	3	60	1	5.9E+01	1.8
Stainless steel	3	240	1	9.9E-01	0.0
Stainless steel	3	0	2	1.8E+06	6.2
Stainless steel	3	60	2	6.9E+00	0.8
Stainless steel	3	120	2	9.9E-01	0.0
Stainless steel	3	240	2	9.9E-01	0.0
Stainless steel	3	0	3	5.8E+05	5.8
Stainless steel	3	60	3	5.4E+03	3.7
Stainless steel	3	120	3	9.9E+01	2.0
Stainless steel	3	240	3	9.9E+00	1.0
Stainless steel	3	0	4	1.1E+06	6.0
Stainless steel	3	60	4	4.9E+03	3.7
Stainless steel	3	120	4	5.0E+02	2.7
Stainless steel	3	240	4	9.9E+00	1.0
Tile	4	0	1	1.9E+06	6.3
Tile	4	60	1	9.4E+01	2.0
Tile	4	0	2	2.2E+06	6.3
Tile	4	60	2	3.0E+00	0.5
Tile	4	120	2	4.6E+01	1.7
Tile	4	0	3	5.6E+05	5.8
Tile	4	60	3	9.9E+01	2.0
Tile	4	120	3	9.9E+01	2.0
Tile	4	240	3	9.9E+01	2.0
Tile	4	0	4	6.2E+05	5.8
Tile	4	60	4	9.9E+01	2.0
Tile	4	120	4	9.9E+00	1.0
Tile	4	240	4	9.9E+00	1.0

Appendix 2. Statistical Analysis.

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   WINKS 7.0.9 PROFESSIONAL Edition                          October 11,2017
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   Two-Way Analysis of Variance
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Var 1: 1 = grout; 2 = plastic; 3 = stainless steel; 4 = tile

   Data Summary:Cell means, standard deviation and counts...

   VAR1       |1.00     |2.00     |3.00     |4.00
              ----------------------------------------
   VAR2       |      6.2|     6.25|     6.08|     6.05|
              |      .85|      .31|      .22|      .29|
         0.00|    n = 3|    n = 4|    n = 4|    n = 4|
              ----------------------------------------
              |      4.8|     3.43|      2.5|     1.63|
              |       .4|      .71|      .44|      .75|
        60.00|    n = 3|    n = 4|    n = 4|    n = 4|
              ----------------------------------------
              |     3.85|      1.5|     1.57|     1.57|
              |      .07|      .71|       .4|      .51|
       120.00|    n = 2|    n = 2|    n = 3|    n = 3|
              ----------------------------------------
              |     3.17|      1.0|       .5|      1.5|
              |      .87|       .0|      .58|      .71|
       240.00|    n = 3|    n = 2|    n = 4|    n = 2|
              ----------------------------------------
   VAR1 is a Fixed Factor.  VAR2 is a Fixed Factor.

   Analysis of Variance Table

   Source                S.S.    DF          MS        F       P
   ------------------------------------------------------------------------
-
   Total                241.91   50
      VAR2              162.76    3       54.25    70.00    <.001
      VAR1               25.81    3        8.60    11.10    <.001
      INTERACTION        14.02    9        1.56     2.01    0.068
     Within Cells        27.13   35         .78
   ------------------------------------------------------------------------
-

   If the interaction effect is considered non-significant,
   multiple comparisons of marginal means is appropriate.

   The following multiple comparisons will be performed:

     Marginal means comparisons:
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MAIN EFFECTS (Compare marginal means) - VAR1 Number of levels = 4

VAR1 (Marginal means)

Means compared are:

VAR1(Gp) 1 = 1.00	Mean= 4.563636	n =	11
VAR1(Gp) 2 = 2.00	Mean= 3.641667	n =	12
VAR1(Gp) 3 = 3.00	Mean= 2.733333	n =	15
VAR1(Gp) 4 = 4.00	Mean= 2.953846	n =	13

Error term used for comparisons = .78 with 35 d.f.

Newman-Keuls Multiple Comp.	Difference	P	Q	Critical q (.05)	
Mean(1.00)-Mean(3.00) =	1.8303	4	7.407	3.818	*
Mean(1.00)-Mean(4.00) =	1.6098	3	6.312	3.464	*
Mean(1.00)-Mean(2.00) =	0.922	2	3.548	2.873	*
Mean(2.00)-Mean(3.00) =	0.9083	3	3.767	3.464	*
Mean(2.00)-Mean(4.00) =	0.6878	2	2.76	2.873	
Mean(4.00)-Mean(3.00) =	0.2205	2	.935	2.873	

Homogeneous Populations, groups ranked

Gp 1 refers to VAR1 (Marginal means)=1.00
Gp 2 refers to VAR1 (Marginal means)=2.00
Gp 3 refers to VAR1 (Marginal means)=3.00
Gp 4 refers to VAR1 (Marginal means)=4.00

Gp Gp Gp Gp
3 4 2 1

This is a graphical representation of the Newman-Keuls multiple comparisons
test. At the 0.05 significance level, the means of any two groups underscored by the same line are not significantly different.

MAIN EFFECTS (Compare marginal means) - VAR2 Number of levels = 4

VAR2 (Marginal means)

Means compared are:

VAR2(Gp) 1 = 0.00	Mean= 6.139999	n =	15
VAR2(Gp) 2 = 60.00	Mean= 2.973334	n =	15
VAR2(Gp) 3 = 120.00	Mean= 2.01	n =	10
VAR2(Gp) 4 = 240.00	Mean= 1.5	n =	11

Error term used for comparisons = .78 with 35 d.f.

Newman-Keuls Multiple Comp.	Difference	P	Q	Critical q (.05)	
Mean(0.00)-Mean(240.00) =	4.64	4	18.777	3.818	*
Mean(0.00)-Mean(120.00) =	4.13	3	16.251	3.464	*
Mean(0.00)-Mean(60.00) =	3.1667	2	13.931	2.873	*
Mean(60.00)-Mean(240.00) =	1.4733	3	5.962	3.464	*
Mean(60.00)-Mean(120.00) =	0.9633	2	3.791	2.873	*
Mean(120.00)-Mean(240.00) =	0.51	2	1.875	2.873	

Homogeneous Populations, groups ranked

Gp 1 refers to VAR2 (Marginal means)=0.00
Gp 2 refers to VAR2 (Marginal means)=60.00
Gp 3 refers to VAR2 (Marginal means)=120.00
Gp 4 refers to VAR2 (Marginal means)=240.00

Gp Gp Gp Gp
4 3 2 1

This is a graphical representation of the Newman-Keuls multiple comparisons
test. At the 0.05 significance level, the means of any two groups underscored by the same line are not significantly different.