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Validation of Quaternary Ammonia and Hydrogen Peroxide Powder for Control of *Listeria monocytogenes* in Ready-to-eat Meat and Poultry Plants

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BACKGROUND AND OBJECTIVE

The American Meat Institute Foundation (AMIF) conducts *Listeria* control workshops for ready-to-eat (RTE) meat and poultry processors to identify and share best practices for control. Some best practices that are being utilized at RTE processors seem to be working based on environmental monitoring data, however, these processes have not been validated and questions remain regarding their true effectiveness.

The objective of this study was to assess the effectiveness of two chemical interventions, quaternary ammonia (quat) and hydrogen peroxide (H_2O_2) powder, to control *Listeria monocytogenes* in RTE meat and poultry processing facilities. Industry members of the AMIF have identified a research need for validation data on the effectiveness of various intervention strategies for control of *L. monocytogenes* in RTE meat and poultry processing environments. One of the expected outcomes of this work was to understand the effect of moisture level on sanitizer effectiveness.

PROJECT DESCRIPTION

Introduction

Listeria monocytogenes is a pathogenic microorganism which is endemic in plant processing environments. An infectious dose of typically more than 100 viable cells can lead to listeriosis which manifests in 3 forms: encephalitis, septicemia and abortion *(5)*. The Centers for Disease Control and Prevention (CDC) have reported that 11 outbreaks (0.2%), 256 (0.2%) cases of foodborne illness and 38 deaths (43.2%) from 1998 to 2002 were caused by *L. monocytogenes (1)*. Foods implicated in these outbreaks included deli meats, hot dogs, queso and goose liver pate.

The ability of a food to support the growth of *L. monocytogenes* to high numbers is one of the major differences between a high risk food and a low risk food. *L. monocytogenes* can grow in foods with a minimum pH and water activity of 4.4 and 0.92, respectively *(3)*. The optimal growth temperature, as a pathogen, is human body temperature, but it will grow in refrigerated foods at a minimal temperature of 31°F. Post-process contamination of RTE meat and poultry products by *L. monocytogenes* has been identified as a hazard reasonably likely to occur during portioning and packaging of these products. *L. monocytogenes* is a foodborne pathogen that can cause illness when present in a food product that supports growth. Refrigerated foods are of particular concern due to the organisms' ability to multiply at low temperatures.

In 2003, the FSIS published compliance guidelines for the control of *L. monocytogenes* in post-lethality exposed RTE meat and poultry products using three alternatives *(7,9)*. Alternative 3 products rely solely on sanitation and Good Manufacturing Practices (GMP's) for control. *Listeria* will continue to be re-introduced into food processing environments, and failure to control this organism on floors increases the risk that product contact surfaces will test positive *(6)*. Historical environmental sampling data from certain RTE meat and poultry establishments producing Alternative 3 products indicate that intervention strategies, including the use of powdered quat and sole scrubbers in the place of foot baths, have controlled the spread of *L. monocytogenes* in those facilities. These assumptions are based on the environmental data, but have not been validated.

Materials and Methods

Sanitizer

Powdered quaternary ammonia (Ecolab, St. Paul, MN) and hydrogen peroxide (DeVere Company Inc., Janesville, WI) were used for tile treatments in these studies.

Tile

Rough surface industrial floor tiles 5 cm length by 5 cm width (Red TR2, Tufco International**,** Siloam Springs, AR) were used in these studies.

Preparation of Test Microorganisms

A cocktail culture of five strains maintained in the Silliker Food Science Center Culture Collection (FSC-CC) and two quat resistant strains acquired from Dr. Kathariou at North Carolina State University (NCSU) were used in this study. Cultures were prepared from freezer stock by transferring each strain to tryptic soy broth with 0.6% yeast extract (TSBYE) and incubating for 18-24 hours at 35°C. The purity of each strain was verified by streak plating on modified oxford (MOX) plates and incubating for 24 h at 35°C. Typical colonies were confirmed using the Vitek Immuno Diagnostic Assay System (VIDAS) method.

In order to cold adapt the cells, a 100 µl aliquot of each overnight culture was transferred to 10-mL of TSBYE and incubated for 3 days at 15°C. Cultures were pooled to prepare an inoculum which contained approximately equal numbers of cells of each strain.

Strain	Source	FSC-CC #
L. monocytogenes -3b	Environmental meat	501
L. monocytogenes -1/2c	Hot dog/food poisoning	525
L. monocytogenes -1/2b	Environmental meat	528
L. monocytogenes -Scott A	Clinical isolate	2399
L. monocytogenes	Biofilm former	2450
L. monocytogenes	Quat resistant from NCSU	3014
L. monocytogenes	Quat resistant from NCSU	3015

Phase 1. Inactivation of *L. monocytogenes* on floor surface using dry powdered quat and H_2O_2

The objective of these experiments was to understand the effectiveness of dry powdered quat and hydrogen peroxide to inactivate *L. monocytogenes* levels on clean and soiled floor tiles. The fundamental questions to be answered included: how long did it take the chemical to react with the microorganism and inactivate it; was the chemical effective on soiled surfaces?

Clean, soiled, treated and untreated floor tiles were prepared. Tiles were inoculated with a cocktail of *L. monocytogenes* strains at low (10^2 CFU/cm^2) and high (10^4 CFU/cm^2) inoculation levels. One hundred µL of the cocktail of *L. monocytogenes* in sterile water were used for the clean surface inoculation. One hundred µL of the cocktail of *L. monocytogenes* in a 10% non fat dry milk solution were used for the soiled surface inoculation. The inoculum was spread evenly onto individual tiles. Prior to addition of powdered quat or hydrogen peroxide at a level of approximately 0.5g/10cm^2 (1.25 g sanitizer/25 cm^2 tile), inoculated floor samples were held at 7°C overnight for bacterial attachment. The powdered chemicals were added to the floor tiles, and pressed into each tile using mechanical action, to replicate use conditions in RTE facilities. Floor samples were pulled at 0, 1, 2, 4, 8, 24 and 48 h of storage, for a total of 7 pull times. The floor tile samples were analyzed for *L. monocytogenes* levels by rinsing the entire floor tile with 100 ml neutralizing buffer for quat treated samples or 0.02% bovine liver catalase (EMD Chemicals, San Diego, CA) (10) for H_2O_2 treated samples and plating to TSAYE with MOX overlay and incubation at 30°C for 48 h *(2)*. The 0-h results represent the counts of *L. monocytogenes* within 15 min of addition of sanitizers upon completion of the overnight storage at 7°C. The experimental matrix was as follows:

- Clean and untreated (control)
- Soiled and untreated (control)
- Clean and treated with quat

- Soiled and treated with quat
- Clean and treated with H_2O_2
- Soiled and treated with H_2O_2

Phase 2. Inactivation of *L. monocytogenes* on floor surface using powdered quat and H_2O_2 at different moisture levels

The objective of these experiments was to understand the effectiveness of powdered quat and hydrogen peroxide to inactivate *L. monocytogenes* at two moisture levels. The fundamental question to be answered was the dry chemical effective, or did it require water/moisture in order to work? Based on the preliminary trials, portions of 0.3 g powdered sanitizers and meat samples per tile (25 cm^2) were used to simulate the conditions in RTE facilities,

A fully cooked turkey breast product without preservatives was ground and inoculated with a cocktail of *L. monocytogenes* strains at low (10^2 CFU/cm^2) and high (10^4 CFU/cm^2) inoculation levels. This sample (100% turkey breast) was used as Moisture Level 1. The ground turkey breast was combined with water (10% turkey breast and 90% water) to make slurry. The slurry was inoculated with a cocktail of *L. monocytogenes* strains at low (10^2 CFU/cm^2) and high (10^4 CFU/cm^2) inoculation levels and used as Moisture Level 2. Portions of powdered quat and hydrogen peroxide (0.3 g sanitizer/25 cm^2 tile) were pressed (ground) onto individual floor tiles using mechanical action. Inoculated turkey breast samples (0.3 g /25 cm^2 tile) were spread evenly onto tiles using mechanical action.

Inoculated floor tiles were stored at 7°C and pulled at 0, 1, 2, 4, 8, 24 and 48 h of storage, for a total of 7 pull times. At each pull time, the floor tile samples were pressed onto individual floor tiles using mechanical action to simulate conditions in RTE facilities. The floor tile samples were analyzed for *L. monocytogenes* levels by rinsing the entire floor tile with 100 ml neutralizing buffer (quat) or catalase (H_2O_2) and plating to TSAYE with MOX overlay and incubation at 30°C for 48 h *(2)*.The 0-h results represent the counts of *L. monocytogenes* within 15 min of addition of inoculated turkey breast samples. The experimental matrix was as follows:

- Untreated Moisture Level 1 (no sanitizer)
- Untreated Moisture Level 2 (no sanitizer)
- Quat treated Moisture Level 1
- Quat treated Moisture level 2
- H_2O_2 treated Moisture level 1
- H_2O_2 treated Moisture level 2

RESULTS AND DISCUSSION

Phase 1. Inactivation of *L. monocytogenes* on floor surface using dry powdered quat and H_2O_2

The objective of these experiments was to assess the effectiveness of dry powdered quat and hydrogen peroxide against *L. monocytogenes* levels on clean and soiled floor tiles.

Survival of *L. monocytogenes* on Clean and Soiled Surfaces

Floor tiles were inoculated with the cocktail of *L. monocytogenes* at low (390 CFU/cm^2) (Tables 1 and 2) and high (39,000 CFU/cm^2) levels (Tables 3 and 4) to determine the survival rate during storage at 7°C for 48 h. Initial die off was observed for both clean and soiled surfaces. However, the die-off rate of *L. monocytogenes* was greater on clean surface tiles compared to that of soiled surface tiles. This may be attributed the protective effect of the 10% non fat dry milk solution used for the soiled surface inoculation against dehydration during storage.

Quat Application on Clean and Soiled Surfaces

Results of the quat treatments at the low inoculation level (480 CFU/cm^2) for clean and soiled tiles surfaces are shown in Table 5 and 6, respectively. No significant reduction in the number of *L. monocytogenes* was observed during storage at 7°C for 48 h. Similarly, the counts of *L. monocytogenes* for clean and soiled tiles surfaces were not affected the by the quat treatments at the high inoculation level (48,000 CFU/cm^2) during storage (Tables 7 and 8).

H_2O_2 Application on Clean and Soiled Surfaces

Results of the H_2O_2 treatments at the low inoculation level (330 CFU/cm^2) for clean and soiled tiles surfaces are shown in Table 9 and 10, respectively. As the data show, *L monocytogenes* was immediately inactivated upon application of H_2O_2 on the clean surface and after 24 hr of storage on the soiled tile surface. At the high inoculum level (33,000 CFU/cm^2*), L monocytogenes* was totally inactivated by H_2O_2 after 8 h and 24 h of storage of the clean and soiled floor tiles, respectively (Tables 11 and 12).

Phase 2. Inactivation of *L. monocytogenes* on floor surface using powdered quat and H_2O_2 at different moisture levels

The objective of these experiments was to assess the effectiveness of dry powdered quat and hydrogen peroxide against *L. monocytogenes* at two moisture levels. A ground fully cooked turkey breast was used as Moisture Level 1. The same product was combined with water to make a slurry (10% turkey breast and 90% water) and used as Moisture Level 2.

Survival of *L. monocytogenes* at Two Moisture Levels

The counts of *L. monocytogenes* on the control floor tiles without sanitizers remained stable during storage at 7°C for 48 h when inoculated at the low inoculation level with the Moisture Level 1 sample (550 CFU/cm^2) (Table 13) and the Moisture Level 2 sample (620 CFU/cm^2) (Table 14). Similarly, the number of *L. monocytogenes* on the control floor tiles without sanitizers remained stable during storage at 7°C for 48 h when inoculated at the high inoculation level with the Moisture Level 1 sample (44,000 CFU/cm^2) (Table 15) and the Moisture Level 2 sample (57,000 CFU/cm^2) (Table 16).

Efficacy of Quat at Two Moisture Levels

At the low inoculation level, dry powdered quat was effective in reducing the number of *L. monocytogenes* on the floor tiles to below the detection limit of 0.4 CFU/cm^2 after 24 h storage at 7°C when inoculated with the Moisture level 1 sample (440 CFU/cm^2) (Table 17). The Moisture Level 2 trials (640 CFU/cm^2) resulted in less than detectable levels (<0.4 CFU/cm^2) of *L. monocytogenes o*n the floor tiles upon application of dry powdered quat (Table 18). Similarly, at the high inoculation level, dry powdered quat was effective in reducing the number of *L. monocytogenes* on the floor tiles to below the detection limit of 0.4 CFU/cm^2 after 24 h storage at 7°C when inoculated with the Moisture level 1 sample (42,000 CFU/cm^2) (Table 19). The Moisture Level 2 trials (59,000 CFU/cm^2) resulted in less than detectable levels (<0.4 CFU/cm^2) of *L. monocytogenes o*n the floor tiles upon application of dry powdered quat (Table 20).

Efficacy of H_2O_2 at Two Moisture Levels

At the low inoculation level, dry powdered H_2O_2 was effective in reducing the number of *L. monocytogenes* on the floor tiles to below the detection limit of 0.4 CFU/cm^2 after 2 h storage at 7°C when inoculated with the Moisture level 1 sample (460 CFU/cm^2) (Table 21). The Moisture Level 2 trials (770 CFU/cm^2) resulted in less than detectable levels (<0.4 CFU/cm^2) of *L. monocytogenes o*n the floor tiles upon application of dry powdered quat (Table 22). Similarly, at the high inoculation level, dry powdered H_2O_2 was effective in reducing the number of *L. monocytogenes* on the floor tiles to below the detection limit of 0.4 CFU/cm^2 after 8 h storage at 7°C when inoculated with the Moisture level 1 sample (57,000 CFU/cm^2) (Table 23). The Moisture Level 2 trials (90,000 CFU/cm^2) resulted in less than detectable levels (<0.4 CFU/cm^2) of *L. monocytogenes o*n the floor tiles upon application of dry powdered quat (Table 24).

Conclusions

- The survival of *L. monocytogenes* on the floor tiles was influenced by the presence organic components
- Quat was not effective against *L. monocytogenes* on clean or soiled dry surfaces
- Quat was influenced by moisture and required moisture to become effective against *L. monocytogenes*
- The efficacy of quat increased with increase in moisture level
- H_2O_2 was effective against *L. monocytogenes* on clean or soiled dry surfaces
- The efficacy of H_2O_2 was more significant in the presence of moisture
- Overall, H_2O_2 was more effective against *L. monocytogenes* under similar conditions compared to quat.

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Table 1. Counts of *L. monocytogenes* on clean and untreated (control) floor tile (CFU/cm^2)- Low inoculum (390 CFU/cm^2)

	DI Water Inoculum		
Time	Replicate 1	Replicate 2	Replicate 3
0 h	4.4	1.6	<0.4
1 h	<0.4	2	0.4
2 h	0.4	0.8	1.6
4 h	<0.4	24	24
8 h	6.4	<0.4	0.8
24 h	<0.4	0.4	<0.4
48 h	<0.4	<0.4	<0.4

Table 2. Counts of *L. monocytogenes* on soiled and untreated (control) floor tile (CFU/cm^2)- Low inoculum (390 CFU/cm^2)

	10% NFDM Inoculum		
Time	Replicate 1	Replicate 2	Replicate 3
0 h	6	10	32
1 h	24	16	20
2 h	24	40	52
4 h	44	20	56
8 h	52	40	48
24 h	48	35	26
48 h	5	1	12

Table 3. Counts of *L. monocytogenes* on clean and untreated (control) floor tile (CFU/cm^2)- High inoculum (39,000 CFU/cm^2)

	DI Water Inoculum		
Time	Replicate 1	Replicate 2	Replicate 3
0 h	160	480	68
1 h	920	36	320
2 h	440	120	360
4 h	290	240	680
8 h	390	330	190
24 h	200	140	250
48 h	280	28	380

Table 4. Counts of *L. monocytogenes* on soiled and untreated (control) floor tile (CFU/cm^2)- High inoculum (39,000 CFU/cm^2)

	10% NFDM Inoculum		
Time	Replicate 1	Replicate 2	Replicate 3
0 h	2800	1400	1800
1 h	9600	4800	4400
2 h	3400	4400	2100
4 h	4000	4800	6400
8 h	2800	6000	6800
24 h	4000	8400	5200
48 h	2000	600	1400

Table 5. Counts of *L. monocytogenes* on clean and Quat treated floor tile (CFU/cm^2)- Low inoculum (480 CFU/cm^2)

	DI Water Inoculum		
Time	Replicate 1	Replicate 2	Replicate 3
0 h	<0.4	<0.4	0.4
1 h	<0.4	<0.4	<0.4
2 h	0.4	<0.4	<0.4
4 h	1.2	<0.4	<0.4
8 h	<0.4	0.8	<0.4
24 h	<0.4	<0.4	<0.4
48 h	<0.4	<0.4	<0.4

Table 6. Counts of *L. monocytogenes* on soiled and Quat treated floor tile (CFU/cm^2)- Low inoculum (480 CFU/cm^2)

	10% NFDM Inoculum		
Time	Replicate 1	Replicate 2	Replicate 3
0 h	116	64	72
1 h	26	28	18
2 h	7	7	15
4 h	23	14	16
8 h	10	16	40
24 h	14	20	20
48 h	52	52	32

Table 7. Counts of *L. monocytogenes* on clean and Quat treated floor tile (CFU/cm^2)- High inoculum (48,000 CFU/cm^2)

	DI Water Inoculum		
Time	Replicate 1	Replicate 2	Replicate 3
0 h	68	52	220
1 h	400	130	400
2 h	260	350	240
4 h	72	92	250
8 h	180	140	200
24 h	130	160	170
48 h	44	40	8

Table 8. Counts of *L. monocytogenes* on soiled and Quat treated floor tile (CFU/cm^2)- High inoculum (48,000 CFU/cm^2)

	10% NFDM Inoculum		
Time	Replicate 1	Replicate 2	Replicate 3
0 h	4800	7200	4800
1 h	2600	3700	3200
2 h	800	600	600
4 h	1300	1700	1600
8 h	2900	2700	2300
24 h	1900	1600	1400
48 h	4400	5200	5200

Table 9. Counts of *L. monocytogenes* on clean and hydrogen peroxide (H_2O_2) treated floor tile (CFU/cm^2) - Low inoculum (330 CFU/cm^2)

	DI Water Inoculum		
Time	Replicate 1	Replicate 2	Replicate 3
0 h	<0.4	<0.4	<0.4
1 h	<0.4	<0.4	<0.4
2 h	<0.4	<0.4	<0.4
4 h	<0.4	<0.4	<0.4
8 h	<0.4	<0.4	<0.4
24 h	<0.4	<0.4	<0.4
48 h	<0.4	<0.4	<0.4

Table 10. Counts of *L. monocytogenes* on soiled and hydrogen peroxide (H_2O_2) treated floor tile (CFU/cm^2)- Low inoculum (330 CFU/cm^2)

	10% NFDM Inoculum		
Time	Replicate 1	Replicate 2	Replicate 3
0 h	46	100	55
1 h	140	48	100
2 h	32	21	26
4 h	1	0.4	<0.4
8 h	<0.4	<0.4	4
24 h	<0.4	<0.4	<0.4
48 h	<0.4	<0.4	<0.4

Table 11. Counts of *L. monocytogenes* on clean and hydrogen peroxide (H_2O_2) treated floor tile (CFU/cm^2)- High inoculum (33,000 CFU/cm^2)

	DI Water Inoculum		
Time	Replicate 1	Replicate 2	Replicate 3
0 h	84	12	76
1 h	72	24	56
2 h	4	8	136
4 h	4	<4	<4
8 h	<4	<4	<4
24 h	<4	<4	<4
48 h	<4	<4	<4

Table 12 . Counts of *L. monocytogenes* on soiled and hydrogen peroxide (H_2O_2) treated floor tile (CFU/cm^2)- High inoculum (33,000 CFU/cm^2)

	10% NFDM Inoculum		
Time	Replicate 1	Replicate 2	Replicate 3
0 h	10000	11000	14000
1 h	14000	18000	10000
2 h	12000	16000	18000
4 h	2800	2300	1200
8 h	370	340	260
24 h	<4	<4	<4
48 h	<4	<4	<4

Table 13**.** Counts of *L. monocytogenes* on untreated (control) floor tile when inoculated with Moisture Level 1 (turkey breast) sample (CFU/cm^2) - Low inoculum (550 CFU/cm^2)

	Moisture Level 1 (turkey breast)		
Time	Replicate 1	Replicate 2	Replicate 3
0 h	560	380	600
1 h	560	680	640
2 h	560	520	840
4 h	560	720	520
8 h	520	440	520
24 h	390	250	680
48 h	480	330	320

Table 14**.** Counts of *L. monocytogenes* on untreated (control) floor tile when inoculated with Moisture Level 2 (10% turkey breast and 90% water) sample (CFU/cm^2) - Low inoculum (620 CFU/cm^2)

	Moisture Level 2 (10% turkey breast and 90% water).		
Time	Replicate 1	Replicate 2	Replicate 3
0 h	600	480	520
1 h	720	560	560
2 h	640	720	680
4 h	480	300	370
8 h	520	520	760
24 h	100	680	600
48 h	250	120	110

Table 15. Counts of *L. monocytogenes* on untreated (control) floor tile when inoculated with Moisture Level 1 (turkey breast) sample (CFU/cm^2) - High inoculum (44,000 CFU/cm^2)

	Moisture Level 1 (turkey breast)		
Time	Replicate 1	Replicate 2	Replicate 3
0 h	38000	39000	48000
1 h	48000	52000	48000
2 h	40000	48000	52000
4 h	28000	36000	35000
8 h	44000	56000	30000
24 h	34000	44000	28000
48 h	17000	21000	24000

Table 16. Counts of *L. monocytogenes* on untreated (control) floor tile when inoculated with Moisture Level 2 (10% turkey breast and 90% water) sample (CFU/cm^2) - High inoculum (57,000 CFU/cm^2)

	Moisture Level 2 (10% turkey breast and 90% water).		
Time	Replicate 1	Replicate 2	Replicate 3
0 h	48000	39000	52000
1 h	68000	52000	48000
2 h	48000	44000	56000
4 h	52000	56000	60000
8 h	40000	52000	23000
24 h	38000	40000	44000
48 h	27000	31000	8800

Table 17**.** Counts of *L. monocytogenes* on Quat treated floor tile when inoculated with Moisture Level 1 (turkey breast) sample (CFU/cm^2) - Low inoculum (440 CFU/cm^2)

	Moisture Level 1 (turkey breast)		
Time	Replicate 1	Replicate 2	Replicate 3
0 h	60	110	53
1 h	8.4	31	47
2 h	2	10	15
4 h	7.2	0.8	1.2
8 h	12	<0.4	0.8
24 h	<0.4	<0.4	<0.4
48 h	<0.4	<0.4	<0.4

Table 18**.** Counts of *L. monocytogenes* on Quat treated floor tile floor tile when inoculated with Moisture Level 2 (10% turkey breast and 90% water) sample (CFU/cm^2) - Low inoculum (640 CFU/cm^2)

	Moisture Level 2 (10% turkey breast and 90% water).		
Time	Replicate 1	Replicate 2	Replicate 3
0 h	<0.4	<0.4	<0.4
1 h	<0.4	<0.4	<0.4
2 h	<0.4	<0.4	<0.4
4 h	<0.4	<0.4	<0.4
8 h	<0.4	<0.4	<0.4
24 h	<0.4	<0.4	<0.4
48 h	<0.4	<0.4	<0.4

Table 19. Counts of *L. monocytogenes* on Quat treated floor tile floor tile when inoculated with Moisture Level 1 (turkey breast) sample (CFU/cm^2) - High inoculum (42,000 CFU/cm^2)

	Moisture Level 1 (turkey breast)		
Time	Replicate 1	Replicate 2	Replicate 3
0 h	6000	12000	10000
1 h	3600	4000	3500
2 h	4800	6000	3100
4 h	1200	260	350
8 h	640	48	1400
24 h	<4	<4	<4
48 h	<4	<4	<4

Table 20. Counts of *L. monocytogenes* on Quat treated floor tile when inoculated with Moisture Level 2 (10% turkey breast and 90% water) sample (CFU/cm^2) - High inoculum (59,000 CFU/cm^2)

	Moisture Level 2 (10% turkey breast and 90% water).		
Time	Replicate 1	Replicate 2	Replicate 3
0 h	<4	<4	<4
1 h	<4	<4	<4
2 h	<4	<4	<4
4 h	<4	<4	<4
8 h	<4	<4	<4
24 h	<4	<4	16
48 h	<4	<4	<4

Table 21. Counts of *L. monocytogenes* on hydrogen peroxide (H_2O_2) treated floor tile when inoculated with Moisture Level 1 (turkey breast) sample (CFU/cm^2) - Low inoculum (460 CFU/cm^2)

	Moisture Level 1 (turkey breast)		
Time	Replicate 1	Replicate 2	Replicate 3
0 h	290	230	290
1 h	2.8	0.4	<0.4
2 h	<0.4	<0.4	<0.4
4 h	<0.4	<0.4	<0.4
8 h	<0.4	<0.4	<0.4
24 h	<0.4	<0.4	<0.4
48 h	<0.4	<0.4	<0.4

Table 22. Counts of *L. monocytogenes* on hydrogen peroxide (H_2O_2) treated floor tile floor tile when inoculated with Moisture Level 2 (10% turkey breast and 90% water) sample (CFU/cm^2) - Low inoculum (770 CFU/cm^2)

	Moisture Level 2 (10% turkey breast and 90% water).		
Time	Replicate 1	Replicate 2	Replicate 3
0 h	<0.4	<0.4	<0.4
1 h	<0.4	<0.4	<0.4
2 h	0.8	<0.4	<0.4
4 h	<0.4	<0.4	<0.4
8 h	<0.4	<0.4	<0.4
24 h	<0.4	<0.4	<0.4
48 h	<0.4	<0.4	<0.4

Table 23**.** Counts of *L. monocytogenes* on hydrogen peroxide (H_2O_2) treated floor tile floor tile when inoculated with Moisture Level 1 (turkey breast) sample (CFU/cm^2) - High inoculum (57,000 CFU/cm^2)

	Moisture Level 1 (turkey breast)		
Time	Replicate 1	Replicate 2	Replicate 3
0 h	3400	200	220
1 h	640	28	920
2 h	20	64	150
4 h	160	8	<4
8 h	<4	<4	<4
24 h	<4	<4	<4
48 h	<4	<4	<4

Table 24. Counts of *L. monocytogenes* on hydrogen peroxide (H_2O_2) treated floor tile when inoculated with Moisture Level 2 (10% turkey breast and 90% water) sample (CFU/cm^2) - High inoculum (90,000 CFU/cm^2)

	Moisture Level 2 (10% turkey breast and 90% water).		
Time	Replicate 1	Replicate 2	Replicate 3
0 h	<4	<4	<4
1 h	<4	<4	<4
2 h	<4	<4	<4
4 h	<4	<4	<4
8 h	<4	<4	<4
24 h	<4	<4	<4
48 h	<4	<4	<4