Validation of the Ultimate Small Area air and surface purification unit against surface contamination of *Listeria monocytogenes* and *Listeria innocua*

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Contents

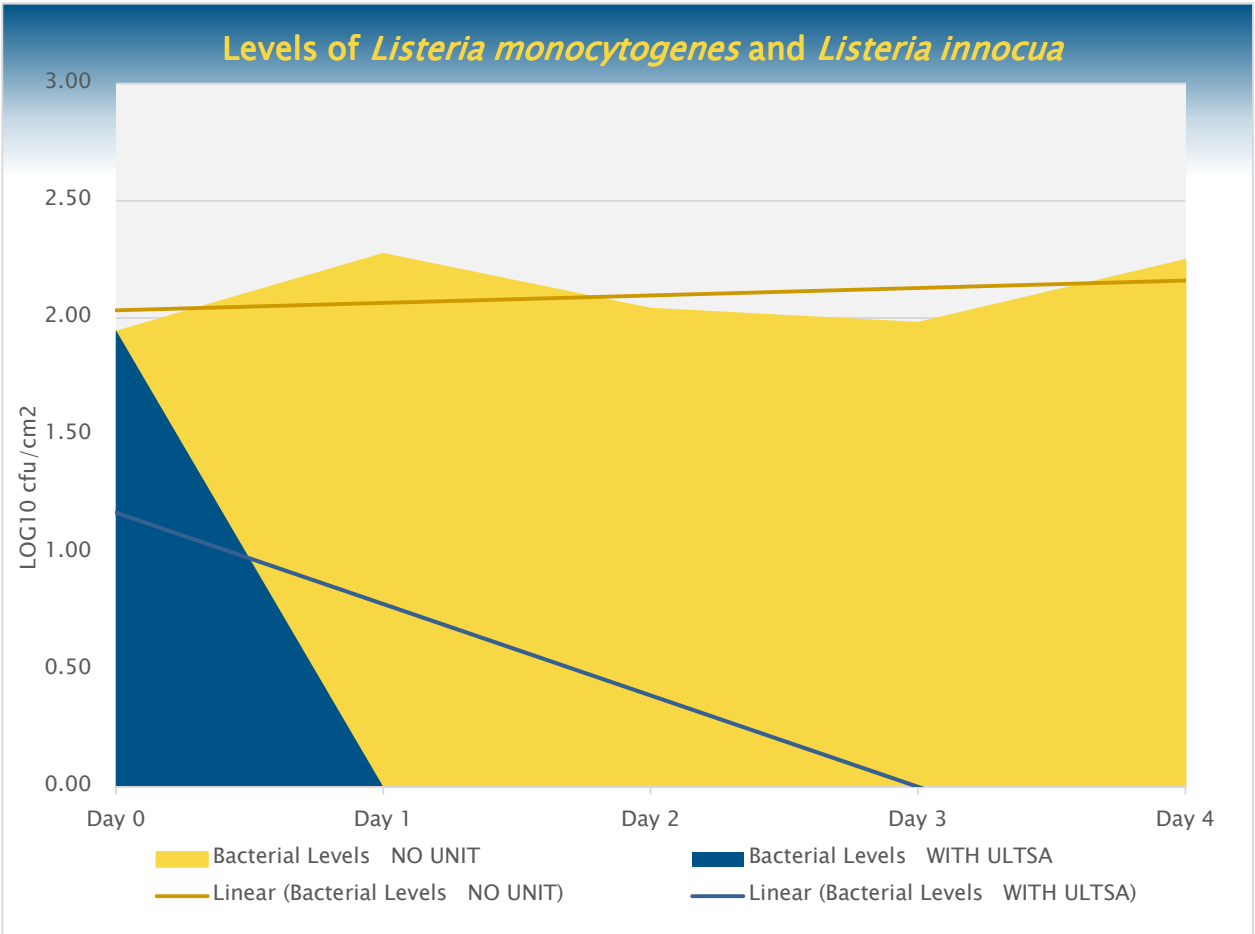
1. Executive Summary

A comparative trial was performed to assess the effect of an Ultimate Small Area air and surface purification unit on surfaces contaminated by two commonly isolated *Listeria* species.

Sterile surfaces were inoculated with a mixture of *Listeria monocytogenes* and *Listeria innocua*, left to dry naturally and stored in a cold room at 1±1°C for four days with the bacterial levels measured daily. The process was performed initially without and the repeated with the Ultimate Small Area unit.

From initial levels of ~2 Log cfu/cm² the trial recorded:

- WITHOUT THE UNIT: steady surface contamination levels with no significant variations but an indication of moderate growth
- WITH THE ULTIMATE SMALL AREA UNIT: a decrease to 0 log cfu/cm² within 24 hours and depletion of the bacterial contamination beyond detectable levels by Day 3



2. Rationale and Overview

Listeria monocytogenes is a bacterial pathogen that is widely distributed in nature. All *Listeria* species are pyschrotrophic and can tolerate high salt as well as a wide pH range. The organism has been isolated from many raw agricultural products, raw meat and poultry products, raw milk, and raw aquaculture products. *Listeria monocytogenes* has been associated with a number of foodborne outbreaks in a variety of refrigerated food products, such as ready-to-eat (RTE), meat, dairy products, processed vegetables as well as fish and seafood [(1) (2) (3)].

The presence of *Listeria* spp. in RTE products is generally known to occur because; 1) there is no lethality step or an insufficient lethality step, so that incoming materials do not receive a process that would be sufficient to eliminate *Listeria* on outgoing products (e.g., fresh or fresh cut fruit and vegetables); 2) products are intended to undergo a listericidal treatment but are processed incorrectly (e.g., an insufficient thermal process); or 3) the product is exposed to the processing environment, and has been contaminated or recontaminated by from the processing environment.

A number of the earliest listeriosis outbreaks in the US (late 1990s, early 2000s) were associated with frankfurters, deli meats and other ready-to-eat (RTE) meat products [(1)]. A 2003 risk assessment conducted by the US Food and Drug Administration (FDA) and US Department of Agriculture Food Safety Inspection Service (USDA FSIS) identified deli meats as the food category most often associated with listeriosis (as compared to other RTE foods such as soft cheeses, and smoked seafood) [(3)]. Due to the early association of listeriosis with RTE meat, the US meat industry was among the first to implement an industry wide program to address the presence on Listeria spp. in the processing environment and on product contact surfaces (PCS, also called food contact surface) as a verification tool to ensure that control programs were effective in preventing potential cross-contamination of finished products. Through collaborative efforts between food companies, industry associations, and regulatory agencies, industry was able to aggressively pursue a 'seek and destroy' approach to identify possible harborage site(s) of the organism [(4)]. Recent data published by the Centers for Disease Control and Prevention (CDC) [(1)] shows that there has been only one outbreak involving *L. monocytogenes* contamination of RTE meat (associated with hogs head cheese, 2010) since this approach has been implemented. Although a number of factors have contributed to this outcome, such as formulating products to prevent the growth of *Listeria monocytogenes*, part of this success is attributed to the allowance for taking corrective actions without holding/implicating/recalling product in reaction to an isolated occurrence of positive Listeria spp. on PCS.

To minimize listeriosis associated with at-risk foods, food manufacturers should consider processes and/or formulations designed to prevent growth of *L. monocytogenes* in the finished product. This could be accomplished by a number of techniques such as frozen distribution, post-packaging treatment, the addition of antimicrobials, etc.[4]

The use of air quality and/or environmental control systems which produce oxygen species by photocatalytic oxidation, has been shown to reduce environmental and surface levels of bacteria. [5]

The client has requested that a validation project is completed on an Ultimate Small Area air and surface purification unit (referred to throughout this report as ULTSA) to assess the effect it has on *Listeria monocytogenes* and *Listeria innocua* surface contamination levels.

To this effect the trial was run in two phases, identical but for the introduction of the ULTSA, each lasting a total of four days:

- Phase 1 was the portion of the trial run without the ULTSA
- Phase 2 was the portion of the trial run with the ULTSA

Sterile surfaces were contaminated by a cocktail of the National Collection of Type Cultures (NCTC) organisms listed below. These were chosen to cover the most commonly isolated members of the *Listeria* Genus.

The inoculated surfaces were then placed inside a temperature monitored cold room at 1±1°C for a total of four days with the bacterial levels measured every 24 hours for each of the two phases of the trial.

Organism
Listeria monocytogenes
Listeria innocua

Table 1 – *table listing the organisms used to contaminate surfaces used in the trial*

This report details the validation of the ULTSA, performed at ALS Rotherham, which operates under ISO 17025 regulations. The project was carried out between in October/November 2018 on the request from Brian Dewsbury, on behalf of Airscience Technology International Limited.

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3. Analytical Procedure

3.1 Organism selection, inoculum preparation and calculation

The inoculum is the mix of organisms used to artificially contaminate the test surface. To ensure the validation study encompasses natural species variation, good laboratory practice prescribes the use of at least two different species of the target microorganism wherever possible. The organisms also need to be adapted to the matrix to be inoculated and its test conditions. Lastly the bacterial levels have to be calculated to allow accurate calibration of the concentration of the inoculum and its volume has to be modulated through the use of serial dilutions before the actual inoculation of the test samples takes place. This is to ensure the maintenance of practical experimental conditions as well as accurate replication of conditions during the intended use of the product tested.

3.1.1 Organism selection

In preparation for the validation trial an appropriate inoculum, quantified, conditioned and of a suitable volume had to be prepared. In regards to the organisms employed in the preparation of the inoculum, a mix was prepared using two National Collection of Type Cultures (NCTC) strains.

Organism	Origin	NCTC reference
Listeria monocytogenes	Type Strain	10527
Listeria innocua	Type Strain	11288

Table 2 – *details of the strain of* Listeria *used for the trial*

3.1.2 Inoculum preparation

The organisms listed above are routinely stored in the Specialist Microbiology Team's organism bank on cryogenic beads. After selecting the suitable organisms for the test, for each a cryogenic bead was placed in a 10ml tube of brain heart infusion (BHI) and left to grow for 24h at 37°C. The following day all organisms were subjected to a serial 1:10 dilution up to 10^{-8} in maximum recovery diluent (MRD) 5ml tubes and the sequential dilutions plated out on nutrient and Columbia horse blood agars (NA and CBA). A 1ml aliquot of the inoculated BHI was also passaged in a fresh BHI tube. BHI tubes and agar plates were then grown at 37°C for 24h. The following day the plates were assessed for counts. The whole process was then repeated for the following three days to stabilise the growth phase of the organisms and to have statistically relevant data in regards to the bacterial levels. This afforded a greater degree of certainty when coming to the preparation of the final inoculum.

3.1.3 Inoculum calculation

Calculations were performed to establish the dilution and volumes required to achieve the desired inoculation levels. Due to the inoculum needing to be placed and dried on the test surface, an arbitrary limit of 10 µl/cm^2 was chosen, this required optimisation of the dilutions used for the preparation of the main inoculum.

The levels of the organisms required for the preparation of the inoculum were assessed against both the typical levels found present during routine environmental testing (to replicate the typical naturally occurring levels) balanced against the requirement to have a span large enough to allow for the measure of the effects the ULTSA has on the organisms used.

A level of ~100 cfu/cm^2 was chosen to replicate naturally occurring levels of the organisms.

To prepare the inoculum, aliquots from each of the appropriate dilution MRD tubes were mixed together in a sterile pot and homogenised before the inoculum was aseptically dispensed on the sterile test surface.

3.2 Inoculation of the samples

Once the inoculum had been prepared it was aseptically dispensed onto the sterile surface of a Petri dish (area 28.26 cm^2). This was performed in triplicate for each day of the trial. A total of 15 plates were inoculated as such and left to aseptically dry.

3.3 Cold storage of the samples

The inoculated samples were placed into a cold store with a volume of ~7.5m^3 and maintained at 1±1°C for the duration of the trial. At the end of Phase 1 of the trial and prior to the beginning of Phase 2, the cold room was cleaned and the ULTSA placed in the cold store and activated. The ULTSA was left to run without test surfaces for a period of ~72h to stabilise the environmental conditions.

3.4 Microbiological Testing

During both phases the samples were tested every 24±4h with the first test (Day 0) occurring immediately after the drying of the inoculum to capture the actual levels of the inoculum dispensed on the test surfaces.

The method used for all microbiological testing is listed below

Target Organism	Method	Method Details	ALS method reference
Listeria spp. (enumeration)	Enumeration of *Listeria monocytogenes* and *Listeria* spp. in food, animal feeding stuffs and swabs	Based on BS ENO ISO 11290-2:2017, and Marks & Spencer Manual of Microbiological Methods, June 2015 , Method 4.11	ESGMM321

Table 3 – *The method used for the trial its details and method reference (the method is in routine use and is UKAS accredited)*

The details and method extract for the method used have not been included in this report however they are available on request.

4. Results

4.1 Phase 1

4.1.1 Raw data

Listeria monocytogenes and *Listeria innocua* enumeration levels without unit					
Day of Testing	Temperature (°C)	cfu/plate (28.26 cm²)	cfu/cm²	Selected Average Value	Standard Deviation
Day 0	1.9	2470	8.74E+01	80.09	
		2640	9.34E+01		18.13
		1680	5.94E+01		
Day 1	2.0	5100	1.80E+02	198.16	
		6400	2.26E+02		24.77
		5300	1.88E+02		
Day 2	1.6	3100	1.10E+02	110.88	
		4600	1.63E+02		51.32
		1700	6.02E+01		
Day 3	1.7	2700	9.55E+01	94.36	
		2400	8.49E+01		8.91
		2900	1.03E+02		
Day 4	1.9	4500	1.59E+02	206.42	
		5000	1.77E+02		66.98
		8000	2.83E+02		

Table 4 – *raw counts for Phase 1 of the trial and basic statistical information.*

4.1.2 Calculated Results

Listeria monocytogenes and *Listeria innocua* enumeration levels without unit					
Day of Testing	Temperature (°C)	cfu/cm²	LOG_{10} cfu/cm²	Standard Deviation	Median LOG_{10} cfu/cm²
Day 0	2.0	8.74E+01	1.94		1.94
		9.34E+01	1.97	0.11	
		5.94E+01	1.77		
Day 1	1.7	1.80E+02	2.26		2.27
		2.26E+02	2.36	0.05	
		1.88E+02	2.27		
Day 2	1.5	1.10E+02	2.04		2.04
		1.63E+02	2.21	0.22	
		6.02E+01	1.78		
Day 3	1.9	9.55E+01	1.98		1.98
		8.49E+01	1.93	0.04	
		1.03E+02	2.01		
Day 4	1.8	1.59E+02	2.20		2.25
		1.77E+02	2.25	0.13	
		2.83E+02	2.45		

***Table 5** – Calculated results for Phase 1 based on the raw data including standard industry indicators such as cfu/cm² and LOG cfu/cm²*

4.2 Phase 2

4.2.1 Raw Data

Listeria monocytogenes and *Listeria innocua* enumeration levels with ULTSA					
Day of Testing	t	cfu/plate	cfu/cm²	Selected Average Value	Standard Deviation
Day 0	1.9	2510	8.88E+01	83.75	
		3000	1.06E+02		25.33
		1590	5.63E+01		
Day 1	2.0	30	1.06E+00	0.47	
		0	0.00E+00		0.54
		10	3.54E-01		
Day 2	1.6	10	3.54E-01	0.47	
		10	3.54E-01		0.20
		20	7.08E-01		
Day 3	1.7	0	0.00E+00	0.00	

		0	0.00E+00		0.00
		0	0.00E+00		
Day 4	1.9	0	0.00E+00	0.00	
		0	0.00E+00		0.00
		0	0.00E+00		

Table 6 – *raw counts for Phase 2 of the trial and basic statistical information.*

4.2.2 Calculated Results

Listeria monocytogenes and *Listeria innocua* enumeration levels with ULTSA					
Day of Testing	**t with unit**	**cfu/cm²**	**LOG_{10} cfu/cm²**	**Standard Deviation**	**Median LOG_{10} cfu/cm²**
Day 0	1.9	8.88E+01	1.95		1.95
		1.06E+02	2.03	0.14	
		5.63E+01	1.75		
Day 1	2.0	1.06E+00	0.03		0.00
		0.00E+00	0.00	0.27	
		3.54E-01	-0.45		
Day 2	1.6	3.54E-01	-0.45		0.00
		3.54E-01	-0.45	0.17	
		7.08E-01	-0.15		
Day 3	1.7	0.00E+00	0.00		0.00
		0.00E+00	0.00	0.00	
		0.00E+00	0.00		
Day 4	1.9	0.00E+00	0.00		0.00
		0.00E+00	0.00	0.00	
		0.00E+00	0.00		

Table 7 – *Calculated results for Phase 2 based on the raw data including standard industry indicators such as cfu/cm² and LOG cfu/cm²*

5. Discussion

During a microbiological challenge investigation, median or average levels of the challenge organisms within ±0.5 log_{10} of the initial (Day 0) level are classified as no significant change. Increases of > 0.5log_{10} from the initial level (positive growth potential) are indicative of growth and decreases of >0.5 log_{10} (negative growth potential) are indicative of die-off. The maximum mean difference in levels of the organism is referred to as the Maximum Growth Potential (δ).

Results from both phases are discussed below.

5.1 Phase 1

The raw counts in colony forming units (cfu) obtained from Phase 1 of the trial were tabulated and processed to obtain the cfu/cm² value and subsequently the Log10 cfu/cm². This due to the Log10 cfu/cm² being the standard measurement of bacterial concentration on surfaces.

These results, together with the temperature values recorded during the trial are pictured below.

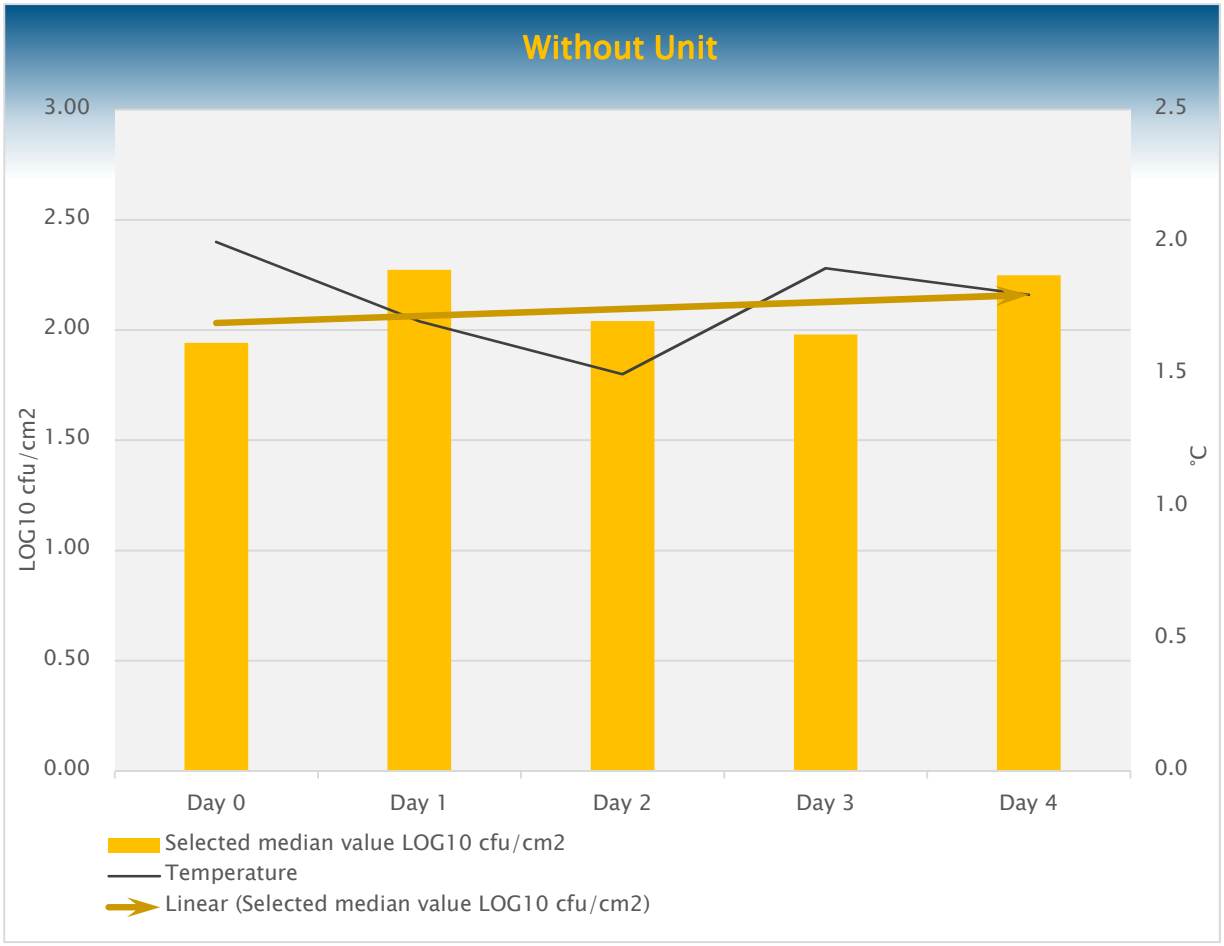

Figure 1 – *Pictorial representation of median values of LOG10 cfu/cm², their trendline and the recorded temperature for Phase 1 of the trial*

Figure 1 illustrates that no difference (Δ) greater than 0.5 Log10 was recorded, indicating no significant change. The greater difference recorded was a positive difference (growth) of 0.33 Log cfu/cm² between Day 0 and Day 1. Although no significant change was recorded, the trend line indication is that of a slow growth trend, which is consistent with *Listeria* species when at refrigerated temperatures[6, 7]. It is also notable that all recorded Δ results are positive, again indicative of growth, albeit not significant.

Analysis of the Δ between Day 0 and the following test Days as a percentage of the Log cfu/cm² value of Day 0 shows again a moderate increase with values ranging from a minimum of 1.99% between Day 0 and Day 3 to a maximum of 16.92% between Day 0 and Day 1 (Table 8).

Listeria monocytogenes and *Listeria innocua* enumeration levels without unit				
Day of Testing	**Average plate counts**	**Median LOG cfu/cm²**	**% Day 0 value**	**Δ in median log_{10} cfu/g between Day 0 and test points**
Day 0	226	1.94	100.00%	
Day 1	560	2.27	116.92%	16.92%
Day 2	313	2.04	105.08%	5.08%
Day 3	267	1.98	101.99%	1.99%
Day 4	583	2.25	115.89%	15.89%

Table 8 – *median Log10 cfu/cm² values and percentage bacterial level expressed as a percentage of Day 0 Log10 cfu/cm² values for Phase 1. Average plate counts are displayed for reference only as the median values are those processed.*

5.2 Phase 2

The raw counts in colony forming units (cfu) obtained from Phase 2 of the trial were tabulated and processed to obtain the cfu/cm² value and subsequently the Log10 cfu/cm².

These results, together with the temperature values recorded during the trial are pictured below.

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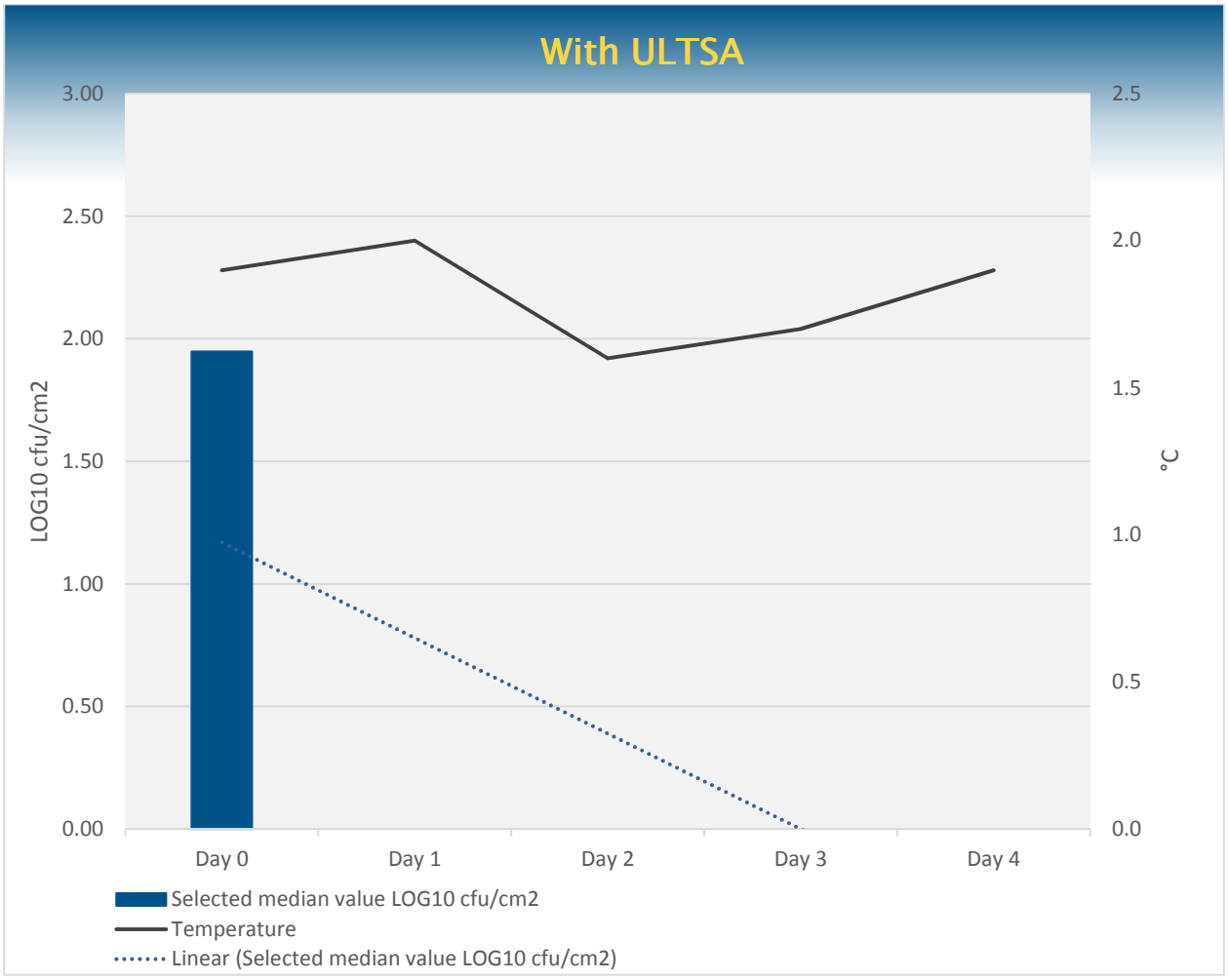

Figure 2 – *Pictorial representation of median values of LOG10 cfu/cm2, their trend line and the recorded temperature for Phase 2 of the trial*

Figure 2 illustrates a recorded Δ of 1.95 Log cfu/cm² between Day 0 and Day 1. As 1.95 Log cfu/cm² equates to 100% of the inoculation levels as recorded on Day 0, no other change was recorded and all subsequent readings were all recorded as 0 Log cfu/cm².

Although counts were recorded on test Days 1 and 2, their cfu/cm² values were values between 0 and 1 (due to the area of the sterile surface being taken into account), equating to a negative Log10 value. For ease of interpretation these values were displayed as 0 as they would during routine accredited analysis of similar surface swab testing.

Analysis of the Δ between Day 0 and the following test Days as a percentage of the Log cfu/cm² value of Day 0 shows a decrease of 100% of the bacterial levels to ≤0 Log cfu/cm² Between Day 0 and Day 1.

The bacterial concentration decreased to beyond detectable levels by Day 3.

Listeria monocytogenes and *Listeria innocua* enumeration levels with ULTSA				
Day of Testing	Average plate counts	Median LOG cfu/cm²	% Day 0 value	Δ in median log_{10} cfu/g between Day 0 and test points
Day 0	237	1.95	100%	
Day 1	1.3	0.00	0%	100%
Day 2	1.3	0.00	0%	0%
Day 3	0	0.00	0%	0%
Day 4	0	0.00	0%	0%

Table 9 – median Log10 cfu/cm² values and percentage bacterial level expressed as a percentage of Day 0 Log10 cfu/cm² values for Phase 2. Average plate counts are displayed for reference only as the median values are those processed.

6. Conclusion

The validation project detailed in this report aimed at assessing the effects of the Ultimate Small Area air and surface purification unit (ULTSA) on a quantified inoculum of *Listeria monocytogenes* and *Listeria innocua*.

In this controlled trial the ULTSA was recorded to cause an abatement of the measurable *Listeria* spp. levels on the test surfaces from an initial level of ~2 Log cfu/cm² to 0 Log cfu/cm² within 24 hours of the introduction of the inoculates surfaces and to beyond detectable levels within 3 Days.

7. References

1. U.S. Centers for Disease Control and Prevention. Morbidity and Mortality Weekly Report. Vital Signs: Listeria Illnesses, Deaths and Outbreaks – United States, 2009–2011. Volume 62, June 7, 2013. [Online] http://www.cdc.gov/mmwr/preview/mmwrhtml/m.

2. Little, C. L., S. K. Sagoo, I. A. Gillespie, K. Grant, and J. McLauchlin. 2009. Prevalence and level of Listeria monocytogenes and other Listeria species in selected retail ready-to-eat foods in the United Kingdom. Journal of Food Protection, Vol. 72, pp. 1869-1877.

3. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Food Safety and Applied Nutrition, and U.S. Department of Agriculture, Food Safety and Inspection Service. 2003. Quantitative assessment of the relative risk to public health from foodborne Listeria monocytogenes among selected categories of ready-to-eat foods. [Online: Cited April 14, 2014]. http://www.fda.gov/Food/ScienceResearch/ResearchAreas/RiskAssessmentSafetyAssessment/ucm1839.

4. adapted from: Brouillette R. *et. al.* 2014. Listeria monocytogenes Guidance on Environmental Monitoring and Corrective Actions in At-risk Foods. Grocery Manufacturers Association.

5. Marsden, J. (2010). Strategies for control of *Listeria monocytogenes*. AFDO, Norfolk, VA (online, cited 18/01/2019) http://www.afdo.org/Resources/Documents/4-news-and-events/past-presentations/1006191100Marsden.pdf

6. Reha O. Azizoglu, J. Osborne, S. Wilson, S. Kathariou, Role of Growth Temperature in Freeze-Thaw Tolerance of Listeria spp., Appl. Environ. Microbiol. Aug 2009, 75 (16) 5315-5320; DOI: 10.1128/AEM.00458-09

7. Yumiko Okada, Izumi Ohnuki, Hodaka Suzuki & Shizunobu Igimi (2013) Growth of Listeria monocytogenes in refrigerated ready-to-eat foods in Japan, Food Additives & Contaminants: Part A, 30:8, 1446-1449, DOI: 10.1080/19440049.2012.762604

8. Final Details

This report was created by Matteo Capocefalo, Technical Project Manager, Specialist Microbiology Team at ALS Rotherham, Units 7&8 Aspen Court, Bessemer Way, Rotherham S60 1FB UK. This report was finalised as dated on the front page of this report and a copy will be held on file.

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