

Confidential Report

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**CHEMICAL FOGGING TRIAL TO ASSESS THE EFFICACY OF DUO MAX
AGAINST SURFACE-ATTACHED BACTERIA**

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INTRODUCTION

Duo Tech Ltd. requested the Food Hygiene Department at Campden Technology Limited (Campden BRI) to assess the efficacy of Duo Max vaporisation fluid, when applied as a chemical fog, against surface-attached methicillin resistant *Staphylococcus aureus* (MRSA), *Escherichia coli*, *Pseudomonas aeruginosa* and *Listeria monocytogenes* using a test protocol used to assess the effectiveness of the chemical and application technique which is based on BS EN 13697: 2001 - Chemical disinfectants and antiseptics - Quantitative non-porous surface test for evaluation of the bactericidal activity and/or fungicidal activity of chemical disinfectants used in food, industrial, domestic and institutional areas.

METHOD

Working subcultures of MRSA (MRSA FH 80/b), *E. coli* (Ec FH 64/g), *P. aeruginosa* (Pa FH 72/i) and *L. monocytogenes* (Lm FH 66/d) were prepared by inoculating slopes of Tryptone Soya Agar (TSA; BS - REC-FH-008) with subcultures derived from the master stock culture. All subcultures were incubated for 24 hours at 37°C. This 1st subculture was used as the working culture and was recovered by adding 5 g of sterile glass beads and 9 ml diluent (BS - REC-FH-004) to each slope. The slopes were then shaken gently to remove the culture from the agar surface. The resultant suspension was filtered through a funnel containing sterile glass wool and eluted with further diluent to maximise recovery. The optical density of each bacterial suspension was measured at 420 nm and calibration graphs of absorbance against viable count were used to determine the concentration. The bacterial suspensions were then diluted with diluent to give an approximate concentration of 10⁸ cfu ml⁻¹.

For the test, 36 stainless steel discs (2 cm diameter, Grade 2 B 1.4301 (EN 10088-1), EN 10 088-2), previously sterilised (in accordance with MA-FH-017 - BS EN 13697:2001), were inoculated with 0.05 ml of the appropriate 10⁸ cfu ml⁻¹ test suspension (9 discs for each test organism). The suspension was dried onto the discs at 37°C for approx. 1 hour. The discs were then allowed to equilibrate to room temperature before the test was commenced. A total of 24 discs (6 for each test organism) were treated with the Duo Max vaporisation fluid fog and 12 discs (3 for each test organism) were left untreated (positive controls).

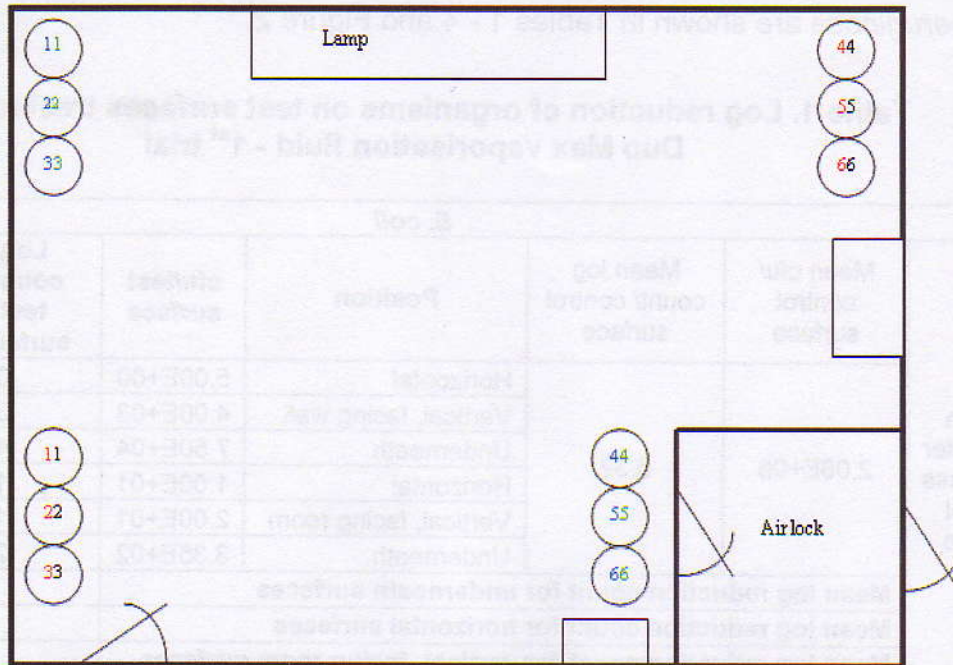
The 24 discs were positioned throughout the air laboratory in three orientations on metal stands: horizontally, vertically and underneath the shelf, as shown in Figure 1. The Neburator fogger was then placed on a stool (61 cm height) in the centre of the room. A 1000 ml predetermined volume of Duo Max neat solution (FH/118758/1) was measured and poured into the Neburator fogger container. 12 ounces of the solution was also poured into the Junior fogger container. For approx. 1-2 minutes, the Junior fogger was used manually to fog difficult to reach areas. The Neburator fogger was then adjusted to give a fine mist and left in the room for a treatment time of 10 minutes. After the treatment, the fogger was switched off and the chemical fog left for a dwell time of one hour. After one hour the extract and supply fans were switched on for 10 minutes to flush the room with fresh air before re-entry.

After treatment the discs were aseptically transferred, using sterile tweezers, from their locations into sterile plastic universal containers (diameter 4 - 5 cm) containing 5 g sterile glass beads (diameter 3 - 4 mm) and 9 ml diluent and 1 ml inactivator (BS - REC-FH-023). The containers were agitated on a horizontal surface for 1 minute to recover the remaining bacteria into suspension. Each sample was serially diluted in diluent to 10^{-4} and plated out in duplicate using TSA. To validate the bacterial recovery process, each disc was recovered from its container and rinsed with 10 ml sterile distilled water (SDW). Each disc was then placed test side up on a pre-poured TSA agar plate. Then 0.1 ml SDW was pipetted onto the disc and rubbed over the surface with a pipette tip for 1 minute. The discs were then over poured with TSA agar. All plates were incubated at 37°C for 48 hours.

The plates were then enumerated and the colony forming units (cfu) per test surface calculated. From the test results and those recorded for the positive controls, the log reduction in bacteria after each treatment was calculated.

The fogging trial was repeated on three separate occasions for each of the test microorganisms.

Figure 1. The arrangement of test surfaces in the air laboratory



Key:

Sample ID	Disc Orientation	Position	Bacterium
1	Horizontal	Top of shelf	MRSA
2	Vertical	Middle shelf: facing wall	MRSA
3	Underneath	Bottom shelf	MRSA
4	Horizontal	Top of shelf	MRSA
5	Vertical	Middle shelf: facing room	MRSA
6	Underneath	Bottom shelf	MRSA
1	Horizontal	Top of shelf	<i>P. aeruginosa</i>
2	Vertical	Middle shelf: facing room	<i>P. aeruginosa</i>
3	Underneath	Bottom shelf	<i>P. aeruginosa</i>
4	Horizontal	Top of shelf	<i>P. aeruginosa</i>
5	Vertical	Middle shelf: facing wall	<i>P. aeruginosa</i>
6	Underneath	Bottom shelf	<i>P. aeruginosa</i>
1	Horizontal	Top of shelf	<i>E. coli</i>
2	Vertical	Middle shelf: facing wall	<i>E. coli</i>
3	Underneath	Bottom shelf	<i>E. coli</i>
4	Horizontal	Top of shelf	<i>E. coli</i>
5	Vertical	Middle shelf: facing room	<i>E. coli</i>
6	Underneath	Bottom shelf	<i>E. coli</i>
1	Horizontal	Top of shelf	<i>L. monocytogenes</i>
2	Vertical	Middle shelf: facing room	<i>L. monocytogenes</i>
3	Underneath	Bottom shelf	<i>L. monocytogenes</i>
4	Horizontal	Top of shelf	<i>L. monocytogenes</i>
5	Vertical	Middle shelf: facing wall	<i>L. monocytogenes</i>
6	Underneath	Bottom shelf	<i>L. monocytogenes</i>

RESULTS

A summary of the results for *E. coli*, *L. monocytogenes*, MRSA and *P. aeruginosa* are shown in Tables 1 - 4 and Figure 2.

Table 1. Log reduction of organisms on test surfaces treated with Duo Max vaporisation fluid - 1st trial

<i>E. coli</i>							
	Mean cfu/ control surface	Mean log count/ control surface	Position	cfu/test surface	Log count/ test surface	Log Reduction	
2 cm diameter stainless steel discs	2.08E+06	6.32	Horizontal	5.00E+00	0.70	5.62	
			Vertical, facing wall	4.00E+03	3.60	2.72	
			Underneath	7.50E+04	4.88	1.44	
			Horizontal	1.00E+01	1.00	5.32	
			Vertical, facing room	2.00E+01	1.30	5.02	
			Underneath	3.35E+02	2.53	3.79	
	Mean log reduction count for underneath surfaces						2.62
	Mean log reduction count for horizontal surfaces						5.47
	Mean log reduction count for vertical, facing room surfaces						5.02
	Mean log reduction count for vertical, facing wall surfaces						2.72
<i>L. monocytogenes</i>							
	Mean cfu/ control surface	Mean log count/ control surface	Position	cfu/test surface	Log count/ test surface	Log Reduction	
2 cm diameter stainless steel discs	1.10E+06	6.04	Horizontal	5.00E+00	0.70	5.50	
			Vertical, facing wall	5.00E+00	0.70	5.50	
			Underneath	4.00E+05	5.60	0.60	
			Horizontal	3.00E+01	1.48	4.72	
			Vertical, facing room	5.00E+00	0.70	5.50	
			Underneath	3.00E+05	5.48	0.72	
	Mean log reduction count for underneath surfaces						0.66
	Mean log reduction count for horizontal surfaces						5.11
	Mean log reduction count for vertical, facing room surfaces						5.50
	Mean log reduction count for vertical, facing wall surfaces						5.50
MRSA							
	Mean cfu/ control surface	Mean log count/ control surface	Position	cfu/test surface	Log count/ test surface	Log Reduction	
2 cm diameter stainless steel discs	3.27E+06	6.51	Horizontal	5.00E+00	0.70	5.81	
			Vertical, facing wall	5.00E+00	0.70	5.81	
			Underneath	6.45E+04	4.81	1.70	
			Horizontal	1.50E+01	1.18	5.33	
			Vertical, facing room	1.50E+01	1.18	5.33	
			Underneath	2.24E+06	6.35	0.16	
	Mean log reduction count for underneath surfaces						0.93
	Mean log reduction count for horizontal surfaces						5.57
	Mean log reduction count for vertical, facing room surfaces						5.33
	Mean log reduction count for vertical, facing wall surfaces						5.81

<i>P. aeruginosa</i>						
	Mean cfu/ control surface	Mean log count/ control surface	Position	cfu/test surface	Log count/ test surface	Log Reduction
2 cm diameter stainless steel discs	2.13E+05	5.33	Horizontal	5.00E+00	0.70	4.63
			Vertical, facing wall	5.00E+00	0.70	4.63
			Underneath	2.60E+05	5.41	-0.08
			Horizontal	1.55E+03	3.19	2.14
			Vertical, facing room	1.12E+05	5.05	0.28
			Underneath	3.30E+05	5.52	-0.19
Mean log reduction count for underneath surfaces						-0.14
Mean log reduction count for horizontal surfaces						3.39
Mean log reduction count for vertical, facing room surfaces						0.28
Mean log reduction count for vertical, facing wall surfaces						4.63

Table 2. Log reduction of organisms on test surfaces treated with Duo Max vapourisation fluid - 2nd trial

<i>E. coli</i>						
	Mean cfu/ control surface	Mean log count/ control surface	Position	cfu/test surface	Log count/ test surface	Log Reduction
2 cm diameter stainless steel discs	1.01E+06	6.00	Horizontal	5.00E+00	0.70	5.30
			Vertical, facing wall	5.80E+02	2.76	3.24
			Underneath	6.05E+03	3.78	2.22
			Horizontal	5.00E+00	0.70	5.30
			Vertical, facing room	2.50E+01	1.40	4.60
			Underneath	1.32E+04	4.12	1.88
Mean log reduction count for underneath surfaces						2.05
Mean log reduction count for horizontal surfaces						5.30
Mean log reduction count for vertical, facing room surfaces						4.60
Mean log reduction count for vertical, facing wall surfaces						3.24
<i>L. monocytogenes</i>						
	Mean cfu/ control surface	Mean log count/ control surface	Position	cfu/test surface	Log count/ test surface	Log Reduction
2 cm diameter stainless steel discs	1.89E+06	6.28	Horizontal	5.00E+00	0.70	5.58
			Vertical, facing wall	2.20E+02	2.34	3.94
			Underneath	7.85E+04	4.89	1.39
			Horizontal	5.00E+00	0.70	5.58
			Vertical, facing room	5.35E+02	2.73	3.55
			Underneath	1.57E+05	5.20	1.08
Mean log reduction count for underneath surfaces						1.24
Mean log reduction count for horizontal surfaces						5.58
Mean log reduction count for vertical, facing room surfaces						3.55
Mean log reduction count for vertical, facing wall surfaces						3.94

MRSA							
	Mean cfu/ control surface	Mean log count/ control surface	Position	cfu/test surface	Log count/ test surface	Log Reduction	
2 cm diameter stainless steel discs	4.29E+06	6.63	Horizontal	5.00E+00	0.70	5.93	
			Vertical, facing wall	1.00E+01	1.00	5.63	
			Underneath	2.33E+06	6.37	0.26	
			Horizontal	5.00E+00	0.70	5.93	
			Vertical, facing room	5.00E+00	0.70	5.93	
			Underneath	1.22E+06	6.09	0.54	
	Mean log reduction count for underneath surfaces						0.40
	Mean log reduction count for horizontal surfaces						5.93
	Mean log reduction count for vertical, facing room surfaces						5.93
	Mean log reduction count for vertical, facing wall surfaces						5.63
<i>P. aeruginosa</i>							
	Mean cfu/ control surface	Mean log count/ control surface	Position	cfu/test surface	Log count/ test surface	Log Reduction	
2 cm diameter stainless steel discs	8.37E+04	4.92	Horizontal	5.00E+00	0.70	4.22	
			Vertical, facing wall	7.60E+02	2.88	2.04	
			Underneath	6.60E+03	3.82	1.10	
			Horizontal	5.00E+00	0.70	4.22	
			Vertical, facing room	1.17E+04	4.07	0.85	
			Underneath	4.80E+05	5.68	-0.76	
	Mean log reduction count for underneath surfaces						0.17
	Mean log reduction count for horizontal surfaces						4.22
Mean log reduction count for vertical, facing room surfaces						0.85	
Mean log reduction count for vertical, facing wall surfaces						2.04	

Table 3. Log reduction of organisms on test surfaces treated with Duo Max vaporisation fluid - 3rd trial

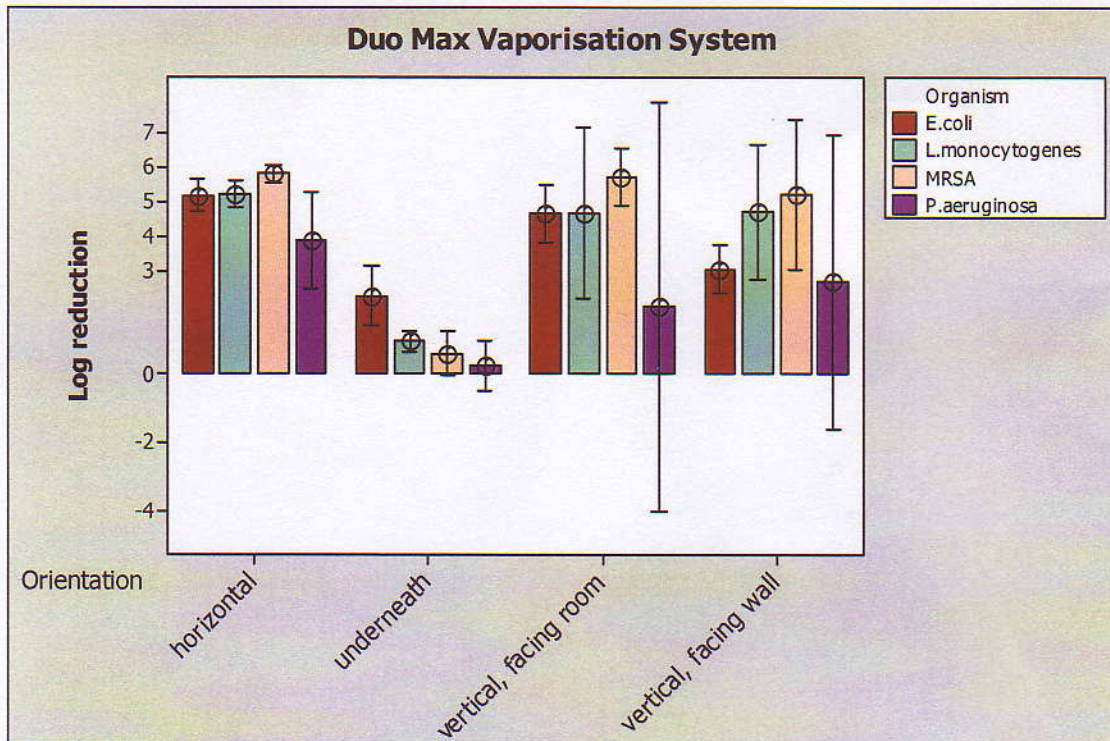
<i>E. coli</i>							
	Mean cfu/ control surface	Mean log count/ control surface	Position	cfu/test surface	Log count/ test surface	Log Reduction	
2 cm diameter stainless steel discs	8.02E+05	5.90	Horizontal	5.00E+00	0.70	5.20	
			Vertical, facing wall	5.45E+02	2.74	3.16	
			Underneath	1.35E+04	4.13	1.77	
			Horizontal	4.00E+01	1.60	4.30	
			Vertical, facing room	3.50E+01	1.54	4.36	
			Underneath	2.70E+03	3.43	2.47	
	Mean log reduction count for underneath surfaces						2.12
	Mean log reduction count for horizontal surfaces						4.75
	Mean log reduction count for vertical, facing room surfaces						4.36
	Mean log reduction count for vertical, facing wall surfaces						3.16

<i>L. monocytogenes</i>						
	Mean cfu/ control surface	Mean log count/ control surface	Position	cfu/test surface	Log count/ test surface	Log Reduction
2 cm diameter stainless steel discs	4.20E+05	5.62	Horizontal	5.00E+00	0.70	4.92
			Vertical, facing wall	1.00E+01	1.00	4.62
			Underneath	4.75E+04	4.68	0.94
			Horizontal	5.00E+00	0.70	4.92
			Vertical, facing room	5.00E+00	0.70	4.92
			Underneath	5.55E+04	4.74	0.88
			Mean log reduction count for underneath surfaces			
Mean log reduction count for horizontal surfaces						4.92
Mean log reduction count for vertical, facing room surfaces						4.92
Mean log reduction count for vertical, facing wall surfaces						9.62
<i>MRSA</i>						
	Mean cfu/ control surface	Mean log count/ control surface	Position	cfu/test surface	Log count/ test surface	Log Reduction
2 cm diameter stainless steel discs	3.85E+06	6.59	Horizontal	5.00E+00	0.70	5.89
			Vertical, facing wall	2.40E+02	2.38	4.21
			Underneath	7.15E+05	5.85	0.74
			Horizontal	5.00E+00	0.70	5.89
			Vertical, facing room	5.00E+00	0.70	5.89
			Underneath	3.30E+06	6.52	0.07
			Mean log reduction count for underneath surfaces			
Mean log reduction count for horizontal surfaces						5.89
Mean log reduction count for vertical, facing room surfaces						5.89
Mean log reduction count for vertical, facing wall surfaces						4.21
<i>P. aeruginosa</i>						
	Mean cfu/ control surface	Mean log count/ control surface	Position	cfu/test surface	Log count/ test surface	Log Reduction
2 cm diameter stainless steel discs	1.95E+06	6.29	Horizontal	5.00E+00	0.70	5.59
			Vertical, facing wall	8.15E+04	4.91	1.38
			Underneath	4.10E+05	5.61	0.68
			Horizontal	7.70E+03	3.89	2.40
			Vertical, facing room	4.00E+01	1.60	4.69
			Underneath	4.45E+05	5.65	0.64
			Mean log reduction count for underneath surfaces			
Mean log reduction count for horizontal surfaces						3.40
Mean log reduction count for vertical, facing room surfaces						1.38
Mean log reduction count for vertical, facing wall surfaces						4.69

Table 4. Summary of mean log reductions of organisms on test surfaces treated with Duo Max vaporisation fluid - 3 trials

Bacterium	Disc orientation	Number of disc sampled during 3 trials	1 st Trial Mean log ₁₀ reduction	2 nd Trial Mean log ₁₀ reduction	3 rd Trial Mean log ₁₀ reduction	Mean log ₁₀ reduction
<i>E. coli</i>	Horizontal	6	5.47	5.30	4.75	5.17
	Underneath	6	2.62	2.05	2.12	2.26
	Vertical, facing room	3	5.02	4.60	4.36	4.66
	Vertical, facing wall	3	2.72	3.24	3.16	3.04
<i>L.monocytogenes</i>	Horizontal	6	5.11	5.58	4.92	5.20
	Underneath	6	0.66	1.24	0.91	0.94
	Vertical, facing room	3	5.50	3.94	4.62	4.69
	Vertical, facing wall	3	5.50	3.55	4.92	4.66
MRSA	Horizontal	6	5.57	5.93	5.89	5.80
	Underneath	6	0.93	0.40	0.41	0.58
	Vertical, facing room	3	5.33	5.93	5.89	5.72
	Vertical, facing wall	3	5.81	5.63	4.21	5.22
<i>P. aeruginosa</i>	Horizontal	6	3.39	4.22	3.40	3.67
	Underneath	6	-0.14	0.17	0.66	0.23
	Vertical, facing room	3	4.63	2.04	1.38	2.68
	Vertical, facing wall	3	0.28	0.85	4.69	1.94

Figure 2. Summary of mean log reductions of organisms on test surfaces treated with Duo Max vaporisation fluid - 3 trials



When applied through the Neburator and Junior fogger, Duo Max achieved:

- a 5.17, 2.26, 4.66 and 3.04 log reduction on horizontal, underneath, vertical, facing room and vertical, facing wall respectively for *E. coli*.
- a 5.20, 0.94, 4.69 and 4.66 log reduction on horizontal, underneath, vertical, facing room and vertical, facing wall respectively for *L. monocytogenes*.
- a 5.80, 0.58, 5.72 and 5.22 log reduction on horizontal, underneath, vertical, facing room and vertical, facing wall respectively for MRSA.
- a 3.67, 0.23, 2.68 and 1.94 log reduction on horizontal, underneath, vertical, facing room and vertical, facing wall respectively for *P. aeruginosa*.

For each of the test bacterium, the mean log₁₀ reduction was not consistent on surfaces at different orientations within the room, with the results consistently being: horizontal > vertical > underneath.

CONCLUSION

Fogging a room with Duo Max vaporisation fluid has shown that it can reduce the number of microorganisms on horizontal surfaces by > 5 log orders for *E. coli*, *L. monocytogenes* and MRSA and > 3.5 log orders for *P. aeruginosa*. The log reduction for each of the test microorganisms was, however, greater on horizontal surfaces than on vertical and underneath surfaces.