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Quantitative Suspension Testing using the OTEX^3 Validated Ozone Generator and JLA HC100 (10KG) Washing Machine.

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QUANTITATIVE SUSPENSION TESTING OF OZONATED WATER USING AN OTEX^3 VALIDATED OZONE DISINFECTION GENERATOR AND JLA HC100 (10KG) WASHING MACHINE.

Introduction

The objective of this study was to demonstrate the bactericidal, fungicidal and sporicidal efficacy of the in-use concentration of Ozone in solution generated continuously by the OTEX^3 Validated Ozone disinfection generator, against a range of typical organisms associated with laundering applications. The study uses the JLA HC100 washing machine as the containment vessel.

The test method used was a Kill Time Assay, whereby the test microbial suspension was introduced directly into the drum of the Washing machine containing a known level of water in the presence of soluble ozone. At various intervals, an aliquot of the test mixture was transferred to a neutralizing solution to inactivate or suppress any bactericidal, fungicidal or sporicidal activity. Using standard microbiological techniques, the number of surviving bacteria, mould spores or bacterial spores was determined and a reduction in viable count calculated.

The study was performed by MGS Laboratories Ltd, an independent contract testing laboratory.

The OTEX^3 Validated Ozone Disinfection Generator and JLA HC 100 washing machines were installed and programmed by JLA representatives.

Experimental Tests and Method:

Preparation of test organisms:
The following organisms were used in the study:

Organism	Source	Number
Pseudomonas aeruginosa	ATCC	15442
MRSA	NCTC	12493
Enterococcus faecalis	NCTC	775
Escherichia coli	NCTC	10418
Acinetobacter baumannii	NCIMB	12457
Aspergillus niger (Spores)	ATCC	16404
Clostridium difficile (Spores)	ATCC	9689

Preparation of Bacteria

Bacteria for testing were cultured onto Tryptone Soy Agar (TSA) for 24 hours before being suspended in Tryptone Soya broth for a further 24 hours.

Preparation of the mould spore suspension

The centre of ten SDA plates were inoculated from the required slope and allowed to grow until the whole plate had sporulated (This was usually not less than 7 days).

The plates were harvested under laminar flow by pipetting 2ml R&P onto the surface of the agar plate, and gently spreading and scraping the diluent over the surface of the plate using a sterile spreader so as to remove as many spores as possible without removing the fungal basal layer or agar.

The resulting suspension of spores/R&P was placed onto a sintered disc 40 micron filter with vacuum flask, and a vacuum generated to pull the liquid and spores through into the flask below.

To aid filtration, the sintered disc was flushed backwards periodically and the suspension above the disc stirred vigorously before re-applying vacuum.

9ml aliquots of R&P were added to the residue, stirred and steps 3 & 4 repeated until the liquid emerging below the disc was no longer black with spores.

The resulting suspension of spores/R&P was then placed into McCartney tubes and centrifuged at 2000rpm for 5mins.

The supernatant was decanted off and the spores re-suspended in purified water. The centrifugation process was repeated until the solution in which the spores were suspended was clear. The suspension was enumerated prior to use.

Immediately before use, the spore suspension was microscopically examined to confirm that less than 1 in 10 fields of view contained mycelial fragments or germinating spores.

If more than 1 in 10 fields of view contained mycelial fragments steps 3 – 8 were repeated.

If more than 1 in 10 fields of view contained germinating spores the suspension was discarded.

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Preparation of Bacterial spores

A Culti-loop™ of *Clostridium difficile* was transferred to 100ml of cooked meat medium and incubated anaerobically at 37°C for not less than 10 days.

The resulting broth was transferred to McCartney tubes and centrifuged at 2000rpm for 20 minutes.

The supernatant was decanted off and the spores resuspended in 50/50 ethanol and purified water and left for 20 minutes.

The centrifugation process was repeated and the supernatant discarded, followed by resuspension of the spores in purified water.

Prior to use, the spore suspension was microscopically examined to confirm that less than 1 in 10 fields of view contained germinating spores.

Preparation of the disinfectant solution:

Ozone was generated in the drum of the JLA HC100 washing machine using the connected OTEX^3 Validated Ozone Disinfection Generator.

Test Parameters

- All tests using bacteria were performed in the presence of the growth medium.
- The fungicidal and sporicidal tests were performed without the presence of the growth medium.
- All tests used multiple contact times of 0, 1, 3, 5, 7, 11 and 15 minutes.
- All testing was performed at 20ºC$\pm$ 2ºC.
- Ozone levels were determined using the Ozone CHEMets® test method.
- Control tests without ozone were conducted for comparison.
- Total Viable counts of the water source were performed to determine if it contained any contaminating organisms that would affect the result.

Test Method

In order to carry out the efficacy tests, the following Washing machine program was used:

Program Details:	Cycle Time (mins)	Dip Level	Temp (C)	Wash Action
Program 1: Cold Wash	30	30cm Volume = 68 litres	Ambient	12secs wash/3secs stop time
Detergent Volumes	No Detergent in use.			

The JLA HC 100 was turned on and allowed to fill with fresh water. Ozone was then injected into the water via a sparger in the machine once a safe water level had been reached.

After the final specified water level had been reached, the test microbial solution was introduced directly in to the drum via the soapbox. This was flushed through with water taken from the drum via the sampling point.

After 30 seconds of mixing, the sampling point was allowed to clear for 15 seconds, and then an aliquot was taken in a sterile container. 1ml of the sample was then transferred to 9 ml of neutralizer and allowed to stand for 5 minutes. This was then diluted down to a countable range suitable for the organism under test and 1ml duplicate pour plates were prepared using the appropriate agar. This first sample was classed as T = 0 and provided the data for the microbial load of the test.

Subsequent samples were taken from the washing machine drum in the same manner after 1, 3, 5, 7, 11 and 15 minutes.

At the same time intervals, the dissolved ozone levels were measured using the Ozone CHEMets® test method which employs DDPD chemistry. A sample is treated with an excess of potassium iodide. Ozone oxidises iodide to iodine. The iodine then oxidises DDPD, a methyl substituted form of DPD (N, N-diethyl-p-phenylenediamine), to form a purple coloured species in direct proportion to the ozone concentration. Results are expressed in ppm.

The bacterial plates were incubated at 30–35°C for 24–48 hours and the fungal plates at 20-25°C for 2-5 days prior to enumeration.

Control tests were also performed without ozone, to determine if the cycle had an effect on antimicrobial activity. These tests were done prior to the OTEX^3 tests to ensure that no residual ozone could affect the result.

Calculation of Results

Determination of Microbial Load

The aliquot taken at T = 0 was used to determine the starting cfu/ml of the test, from which the Log reduction of viable cells could be calculated.

The dilution(s) offering a satisfactory plate count range between 30-300cfu were identified.

N was then calculated using the following equation:

$$N = c/(n \times d)$$

Where: N is cfu/ml of the test inoculum
c is the sum of the colonies counted on the plate set taken into account
n is the number of plates taken into account
d is the dilution factor corresponding to the dilution taken into account

Determination of Antimicrobial efficacy

A dilution factor of log 1 was applicable.

The average plate count was calculated. Where the result = 0.5 this was recorded as 1. Where the result = TNTC this was recorded as >300.

Log_{10} of the average is then calculated

Then (log N -1) – (log test) = log reduction

Where the log (test) is "–" the log reduction was recorded as "greater than" (>).

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Summary of Log reductions

Test 1:

Organism: *Pseudomonas aeruginosa*					
	Test			Control	
Time (mins)	Ozone (ppm)	Log Recovery	Log Reduction	Log Recovery	Log Reduction
0	0.050	6.4		6.3	
1	0.075	6.4	0.0	6.3	0.0
3	0.250	6.3	0.1	6.3	0.0
5	0.350	5.5	0.9	6.3	0.0
7	0.400	3.4	3.0	6.3	0.0
11	0.400	<2.0	>4.4	6.3	0.0
15	0.450	<2.0	>4.4	6.3	0.0

Test 2:

Organism: MRSA					
	Test			Control	
Time (mins)	Ozone (ppm)	Log Recovery	Log Reduction	Log Recovery	Log Reduction
0	0.050	5.7		5.8	
1	0.075	5.8	-0.1	5.8	0.0
3	0.250	4.5	1.2	5.8	0.0
5	0.350	<1.0	>4.7	5.8	0.0
7	0.400	<1.0	>4.7	5.7	0.1
11	0.400	<1.0	>4.7	5.7	0.1
15	0.450	<1.0	>4.7	5.8	0.0

Test 3:

Organism: *Enterococcus faecalis*					
	Test			Control	
Time (mins)	Ozone (ppm)	Log Recovery	Log Reduction	Log Recovery	Log Reduction
0	0.050	6.0		6.1	
1	0.100	5.8	0.2	6.0	0.1
3	0.250	5.5	0.5	6.0	0.1
5	0.450	2.2	3.8	6.0	0.1
7	0.450	1.8	4.2	6.0	0.1
11	0.450	1.6	4.4	6.0	0.1
15	0.450	1.3	4.7	6.0	0.1

Test 4:

Organism: *Escherichia coli*					
	Test			Control	
Time (mins)	Ozone (ppm)	Log Recovery	Log Reduction	Log Recovery	Log Reduction
0	0.050	5.5		5.8	
1	0.075	5.6	-0.1	5.9	-0.1
3	0.250	5.1	0.4	6.0	-0.2
5	0.350	3.5	2.0	5.7	0.1
7	0.400	2.1	3.4	5.9	-0.1
11	0.400	1.6	3.9	5.8	0.0
15	0.450	<1.0	>4.5	5.8	0.0

Test 5:

Organism: *Acinetobacter baumannii*					
	Test			Control	
Time (mins)	Ozone (ppm)	Log Recovery	Log Reduction	Log Recovery	Log Reduction
0	0.050	5.5		5.9	
1	0.100	4.7	0.8	5.9	0.0
3	0.250	4.9	0.6	6.0	-0.1
5	0.450	3.8	1.7	6.0	-0.1
7	0.450	3.5	2.0	6.0	-0.1
11	0.450	3.2	2.3	6.0	-0.1
15	0.450	2.7	2.8	6.0	-0.1

Test 6:

Organism: *Aspergillus niger* (Spores)					
	Test			Control	
Time (mins)	Ozone (ppm)	Log Recovery	Log Reduction	Log Recovery	Log Reduction
0	0.450	3.8		3.8	
1	0.550	3.8	0.0	3.7	0.1
3	0.700	3.0	0.8	3.7	0.1
5	1.000	1.7	2.1	3.7	0.1
7	1.200	1.0	2.8	3.7	0.1
11	1.500	1.0	2.8	3.7	0.1
15	1.800	<1.0	>2.8	3.7	0.1

Test 7:

Organism: *Clostridium difficile* (Spores)					
	Test			Control	
Time (mins)	Ozone (ppm)	Log Recovery	Log Reduction	Log Recovery	Log Reduction
0	0.400	2.6		2.6	
1	0.500	2.4	0.2	2.6	0.0
3	0.750	2.0	0.6	2.6	0.0
5	0.900	1.0	1.6	2.6	0.0
7	1.200	<1.0	>1.6	2.6	0.0
11	1.400	<1.0	>1.6	2.6	0.0
15	1.200	<1.0	>1.6	2.6	0.0

Discussion:

Using the OTEX^3 Validated Ozone generator, a continual reduction of *Pseudomonas aeruginosa* cells occurred as the levels of dissolved ozone increased. After 5 minutes, the dissolved ozone had reached 0.350ppm and achieved a Log 0.9 reduction of viable cells. An increase of 0.050ppm gave an increase in Log reduction of 2.1. After 11 minutes, the dissolved level of ozone had reached 0.450ppm and achieved a log reduction of >4.4. This result was maintained after 15 minutes, with no discernable recovery of organism.

When testing ozone against MRSA, the same increase of dissolved ozone gave a much quicker Log Kill. After 5 minutes, the dissolved ozone level reached 0.350ppm and gave a Log reduction of >4.7.

The viable cells of *Enterococcus faecalis* gave a more gradual decline over the 15 minute contact time, suggesting a greater resistance against Ozone than the previous 2 organisms. After 5 minutes, dissolved ozone levels reached 0.450ppm and the Log reduction achieved was 3.8. Dissolved ozone remained at the same level for the rest of the test and the degree of biocidal activity increased to Log 4.7 after 15 minutes, however viable cells of *E. faecalis* were still recovered.

Testing of OTEX^3 generated ozone at 0.450ppm against *E. coli* gave a >4.5 Log reduction of viable cells after 15 minutes with no discernable recovery of cells. As with the previous 3 organisms, most of the biocidal efficacy occurred after dissolved levels of ozone reached >0.250ppm.

The Log reduction of *Acinetobacter baumannii* cells was much lower than the other 4 bacteria. After 15 minutes, dissolved levels of ozone had reached 0.450ppm, but the log reduction had only reached 2.8 of the potential 4.5 Log reduction. A study conducted by Roberto S. Chamul, Miranda Reed and Juan L. Silva titled 'Chiller Water Treatment from Channel Catfish (Ictalurus punctatus) Processing Plants with Ultrasound, Ozone and Pulsed light', found that 'Of the Gram-negative microorganisms, Acinetobacter baumannii was found to be one of the most resistant to ozone at low concentrations'
http://www.msstate.edu/org/MAS/july02journal/july.PDF

It should be noted that the tests conducted against all the above bacteria were performed in the presence of the growth medium (Tryptone Soya Broth). It is common knowledge that Ozone is readily absorbed by organic materials. This means that the dissolved ozone had to overcome this absorption before it could have a biocidal effect.

Both of the spore forming organisms were tested without growth medium, instead they were introduced into the washing machine drum suspended in sterile water.

In the case of OTEX^3 generated dissolved ozone against *Aspergillus niger* spores, the system showed a gradual increase in biocidal efficacy, under conditions more applicable to the final use of the system. Dissolved ozone levels reached 1.800ppm after 15 minutes giving a Log reduction of >2.8 viable spores.

Due to the limitations of generating enough *A. niger* spores to return a significant inoculum when dosed into the drum. It is difficult to tell if the decrease in viable spores is gradual, due to the fact that some spores are more susceptible than others, or if the system would have demonstrated lower level biocidal efficacy with an increase of organic matter that a higher yield of spores would have produced.

Due to the difficulty in obtaining *Clostridium difficile* spores, the starting challenge in 68 litres of water was again low. In the presence of no interfering substance, the OTEX^3 was able to generate very high levels of dissolved ozone.

After 1 minute, dissolved ozone had reached 0.500ppm which gave a Log 0.2 reduction of Clostridium difficile. After 3 minutes, dissolved ozone reached 0.750ppm giving a Log reduction of 0.6. After reaching 1.200ppm of dissolved ozone after 7 minutes, no discernable *Clostridium difficile* spores were recovered giving a reduction of viable spores of >1.6 logs.

In every instance, the control tests recovered levels of organism that prove the action of the machine has no antimicrobial activity and that the log reductions of the tests are down to the dissolved ozone alone.

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Conclusions:

- In the case of the bacterial tests, the addition of the growth medium reduced the levels that the dissolved ozone could reach.
- Levels of ≤0.250ppm of dissolved ozone had minimal effect on the bacteria. Levels >0.450ppm showed good bactericidal activity except for against *Acinetobacter baumannii*.
- Against fungal spores, dissolved ozone levels of >1.200ppm are required to attain a 2.8 Log reduction.
- For Clostridium difficile spores, a dissolved ozone level of between 0.900ppm-1.200ppm give a >1.6 log reduction with no discernable recovery.

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APPENDIX 1 – DETAILED RESULT SHEETS

Date test Performed:	10/05/07
Product	Ozone
Test Temperature	20°C ± 2 °C
Organism	*Pseudomonas aeruginosa*

Control Test

Contact Time	Dilution	Plate Count			Cfu/ml	Log	Log Reduction
		Plate 1	Plate 2	Av			
0	-4	187	169	178	1.8×10^6	6.3	
1	-4	176	176	176	1.8×10^6	6.3	0.0
3	-4	193	174	184	1.8×10^6	6.3	0.0
5	-4	191	183	187	1.9×10^6	6.3	0.0
7	-4	198	164	181	1.8×10^6	6.3	0.0
11	-4	185	180	183	1.8×10^6	6.3	0.0
15	-4	177	175	176	1.8×10^6	6.3	0.0

Test: Disinfection Level: MAX Pressure (psi): 5 SCFH: 4.25
Ozone activation: 56 seconds Level Stop: 2minutes 24 seconds

Contact Time	Ozone Level (ppm)	Dilution	Plate Count			Cfu/ml	Log	Log Reduction
			Plate 1	Plate 2	Av			
0	0.05	-4	200	191	196	2.0×10^6	6.4	
1	0.075	-4	199	195	197	2.0×10^6	6.4	0.0
3	0.25	-4	198	184	191	1.9×10^6	6.3	0.1
5	0.35	-4	28	36	32	3.2×10^5	5.5	0.9
7	0.40	-2	26	29	28	2.8×10^3	3.4	3.0
11	0.40	-2	Nil	Nil	<1	<100	<2.0	>4.4
15	0.45	-2	Nil	Nil	<1	<100	<2.0	>4.4

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Date test Performed:	10/05/07
Product	Ozone
Test Temperature	20°C ± 2 °C
Organism	*MRSA*

Control Test

Contact Time	Dilution	Plate Count			Cfu/ml	Log	Log Reduction
		Plate 1	Plate 2	Av			
0	-4	64	52	58	5.8×10^5	5.8	
1	-4	63	60	62	6.2×10^5	5.8	0.0
3	-4	69	62	66	6.6×10^5	5.8	0.0
5	-4	54	62	58	5.8×10^5	5.8	0.0
7	-4	52	58	55	5.8×10^5	5.7	0.1
11	-4	55	57	56	5.6×10^5	5.7	0.1
15	-4	55	59	57	5.7×10^5	5.8	0.0

Test: Disinfection Level: MAX Pressure (psi): 5 SCFH: 4.25
Ozone activation: 56 seconds Level Stop: 2minutes 24 seconds

Contact Time	Ozone Level (ppm)	Dilution	Plate Count			Cfu/ml	Log	Log Reduction
			Plate 1	Plate 2	Av			
0	0.05	-4	54	57	56	5.6×10^5	5.7	
1	0.075	-4	58	55	57	5.7×10^5	5.8	-0.1
3	0.25	-3	33	35	34	3.4×10^4	4.5	1.2
5	0.35	-1	Nil	Nil	<1	<10	<1.0	>4.7
7	0.40	-1	Nil	Nil	<1	<10	<1.0	>4.7
11	0.40	-1	Nil	Nil	<1	<10	<1.0	>4.7
15	0.45	-1	Nil	Nil	<1	<10	<1.0	>4.7

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Date test Performed:	17/05/07
Product	Ozone
Test Temperature	20°C ± 2 °C
Organism	*Enterococcus faecalis*

Control Test

Contact Time	Dilution	Plate Count			Cfu/ml	Log	Log Reduction
		Plate 1	Plate 2	Av			
0	-4	134	107	121	1.2×10^6	6.1	
1	-4	98	101	100	1.0×10^6	6.0	0.1
3	-4	105	105	105	1.1×10^6	6.0	0.1
5	-4	112	102	107	1.1×10^6	6.0	0.1
7	-4	110	113	112	1.1×10^6	6.0	0.1
11	-4	100	116	108	1.1×10^6	6.0	0.1
15	-4	101	112	107	1.1×10^6	6.0	0.1

Test: Disinfection Level: MAX Pressure (psi): 5 SCFH: 5.0
Ozone activation: 58 seconds Level Stop: 2minutes 36 seconds

Contact Time	Ozone Level (ppm)	Dilution	Plate Count			Cfu/ml	Log	Log Reduction
			Plate 1	Plate 2	Av			
0	0.05	-4	100	87	94	9.4×10^5	6.0	
1	0.10	-4	63	54	59	5.9×10^5	5.8	0.2
3	0.25	-4	35	30	33	3.3×10^5	5.5	0.5
5	0.45	-1	17	15	16	1.6×10^2	2.2	3.8
7	0.45	-1	7	7	7	7.0×10^1	1.8	4.2
11	0.45	-1	4	4	4	4.0×10^1	1.6	4.4
15	0.45	-1	3	1	2	2.0×10^1	1.3	4.7

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Date test Performed:	10/05/07
Product	Ozone
Test Temperature	20°C ± 2 °C
Organism	Escherichia coli

Control Test

Contact Time	Dilution	Plate Count			Cfu/ml	Log	Log Reduction
		Plate 1	Plate 2	Av			
0	-4	69	65	67	6.7×10^5	5.8	
1	-4	94	68	81	8.1×10^5	5.9	-0.1
3	-4	91	90	91	9.1×10^5	6.0	-0.2
5	-4	45	55	50	5.0×10^5	5.7	0.1
7	-4	71	79	75	7.5×10^5	5.9	-0.1
11	-4	54	63	59	5.9×10^5	5.8	0.0
15	-4	67	73	70	7.0×10^5	5.8	0.0

Test: Disinfection Level: MAX Pressure (psi): 5 SCFH: 4.25
Ozone activation: 56 seconds Level Stop: 2minutes 24 seconds

Contact Time	Ozone Level (ppm)	Dilution	Plate Count			Cfu/ml	Log	Log Reduction
			Plate 1	Plate 2	Av			
0	0.05	-3	327	341	334	3.3×10^5	5.5	
1	0.075	-3	400	365	383	3.8×10^5	5.6	-0.1
3	0.25	-3	136	128	132	1.3×10^5	5.1	0.4
5	0.35	-2	31	34	33	3.3×10^3	3.5	2.0
7	0.40	-1	13	15	14	1.4×10^2	2.1	3.4
11	0.40	-1	4	4	4	4.0×10^1	1.6	3.9
15	0.45	-1	Nil	Nil	Nil	<10	<1.0	>4.5

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Date test Performed:	17/05/07
Product	Ozone
Test Temperature	20°C ± 2 °C
Organism	*Acinetobacter baumannii*

Control Test

Contact Time	Dilution	Plate Count			Cfu/ml	Log	Log Reduction
		Plate 1	Plate 2	Av			
0	-4	85	93	89	8.9×10^5	5.9	
1	-4	85	83	84	8.4×10^5	5.9	0.0
3	-4	110	118	114	1.1×10^6	6.0	-0.1
5	-4	112	86	99	9.9×10^5	6.0	-0.1
7	-4	110	97	104	1.0×10^6	6.0	-0.1
11	-4	121	104	113	1.1×10^6	6.0	-0.1
15	-4	109	111	110	1.1×10^6	6.0	-0.1

Test: Disinfection Level: MAX Pressure (psi): 5 SCFH: 5.0
Ozone activation: 58 seconds Level Stop: 2minutes 36 seconds

Contact Time	Ozone Level (ppm)	Dilution	Plate Count			Cfu/ml	Log	Log Reduction
			Plate 1	Plate 2	Av			
0	0.05	-4	33	29	31	3.1×10^5	5.5	
1	0.10	-3	48	45	47	4.7×10^4	4.7	0.8
3	0.25	-3	75	70	73	7.3×10^4	4.9	0.6
5	0.45	-2	66	62	64	6.4×10^3	3.8	1.7
7	0.45	-2	31	34	33	3.3×10^3	3.5	2.0
11	0.45	-1	158	142	150	1.5×10^3	3.2	2.3
15	0.45	-1	48	42	45	4.5×10^2	2.7	2.8

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Date test Performed:	09/05/07
Product	Ozone
Test Temperature	20°C ± 2 °C
Organism	*Aspergillus niger*

Control Test

Contact Time	Dilution	Plate Count			Cfu/ml	Log	Log Reduction
		Plate 1	Plate 2	Av			
0	-2	57	58	58	5.8×10^3	3.8	
1	-2	55	56	56	5.6×10^3	3.7	0.1
3	-2	57	49	53	5.3×10^3	3.7	0.1
5	-2	50	58	54	5.4×10^3	3.7	0.1
7	-2	59	49	54	5.4×10^3	3.7	0.1
11	-2	60	51	56	5.6×10^3	3.7	0.1
15	-2	39	50	45	4.5×10^3	3.7	0.1

Test: Disinfection Level: MAX Pressure (psi): 5.0 SCFH: 4.50
Ozone activation: 55 seconds Level Stop: 2minutes 25 seconds

Contact Time	Ozone Level (ppm)	Dilution	Plate Count			Cfu/ml	Log	Log Reduction
			Plate 1	Plate 2	Av			
0	0.45	-2	60	65	63	6.3×10^3	3.8	
1	0.55	-2	57	68	63	6.3×10^3	3.8	0.0
3	0.70	-1	119	103	111	1.1×10^3	3.0	0.8
5	1.00	-1	3	6	5	5.0×10^1	1.7	2.1
7	1.20	-1	2	Nil	1	1.0×10^1	1.0	2.8
11	1.50	-1	2	Nil	1	1.0×10^1	1.0	2.8
15	1.80	-1	Nil	Nil	Nil	<10	<1.0	>2.8

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Date test Performed:	18/05/07
Product	Ozone
Test Temperature	20°C ± 2 °C
Organism	*Clostridium difficile*

Control Test

Contact Time	Dilution	Plate Count			Cfu/ml	Log	Log Reduction
		Plate 1	Plate 2	Av			
0	-1	44	39	42	4.2×10^2	2.6	
1	-1	39	37	38	3.8×10^2	2.6	0.0
3	-1	36	38	37	3.7×10^2	2.6	0.0
5	-1	39	39	39	3.9×10^2	2.6	0.0
7	-1	35	48	42	4.2×10^2	2.6	0.0
11	-1	41	43	42	4.2×10^2	2.6	0.0
15	-1	43	35	39	3.9×10^2	2.6	0.0

Test: Disinfection Level: MAX Pressure (psi): 5.0 SCFH: 4.50
Ozone activation: 55 seconds Level Stop: 2minutes 25 seconds

Contact Time	Ozone Level (ppm)	Dilution	Plate Count			Cfu/ml	Log	Log Reduction
			Plate 1	Plate 2	Av			
0	0.40	-1	37	42	40	4.0×10^2	2.6	
1	0.50	-1	25	20	23	2.3×10^2	2.4	0.2
3	0.75	-1	11	7	9	9.0×10^1	2.0	0.6
5	0.90	-1	1	1	1	1.0×10^1	1.0	1.6
7	1.20	-1	Nil	Nil	Nil	<10	<1.0	>1.6
11	1.40	-1	Nil	Nil	Nil	<10	<1.0	>1.6
15	1.20	-1	Nil	Nil	Nil	<10	<1.0	>1.6