Microsearch Laboratories Ltd.

JLA™
Your inhouse Laundry Partner

Bacteriological Investigation:
Otex Laundry System
Solution Test

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1. OBJECTIVE

A laboratory based investigation into the effectiveness of JLA's OTEX system has been carried out, the objective of this work was to provide documentary evidence on the bactericidal activity of the OTEX system against thermal disinfection wash processes. This report documents the work carried out under laboratory conditions on 1st July 2005 at JLA's R & D Technical Laboratory.

2. TEST METHODOLOGY

The following micro organisms were independently prepared for testing by Microsearch Laboratories Ltd:

Solution Challenge Test Organisms	
Micro-organism	**cfu/ml**
Staphylococcus aureus	1.3E+08
Pseudomonas aeruginosa	3.1E+09
Candida Albicans	3.1E+08
Escherichia coli	5.2E+08
Streptococcus faecalis	5.0E+08
Aspergillus niger	3.1E+08
Clostridium difficile	4.2E+08
Clostridium perfringens	9.2E+08
Campylobacter jejuni	6.0E+08
Aeromonas mixed species	8.2E+08
Actinobacter sps	4.3E+08
Lactobacilli sps	3.9E+08
Virus particle	**Particles/ml**
Lambda phage	3.8E+24
FCoVA	2.6E+24
Saccharomyces virus ScV-L-BC	3.1E+23
Vibrio phage fs1	2.6E+28

3. PROGRAMME DETAILS AND TEST CONDITIONS

Tests were carried out on an extended sluice program using a JLA HW164 (16 k dry weight) washing machine. No detergent was employed during this series of tests. Details are given below:

Program Details:	**Cycle Time (mins)**	**Temp (C)**	**Wash Action**
Program 1: Cold Sluice	30	Ambient	12secs wash/3secs stop time
Program 2: Thermal Sluice	30	75C	
Detergent Volumes	No Detergent in use.		

Tests were conducted with water temperatures at both ambient i.e. as supplied and at the recommended thermal disinfection temperature of 75°C. Domestic supply water was employed with a water hardness of 60ppm $CaCO_3$ for all tests. Note no detergent was used throughout the trials.

A single unit OTEX system was employed and was maintained at the following settings throughout the trial with the exception of the control test with no ozone

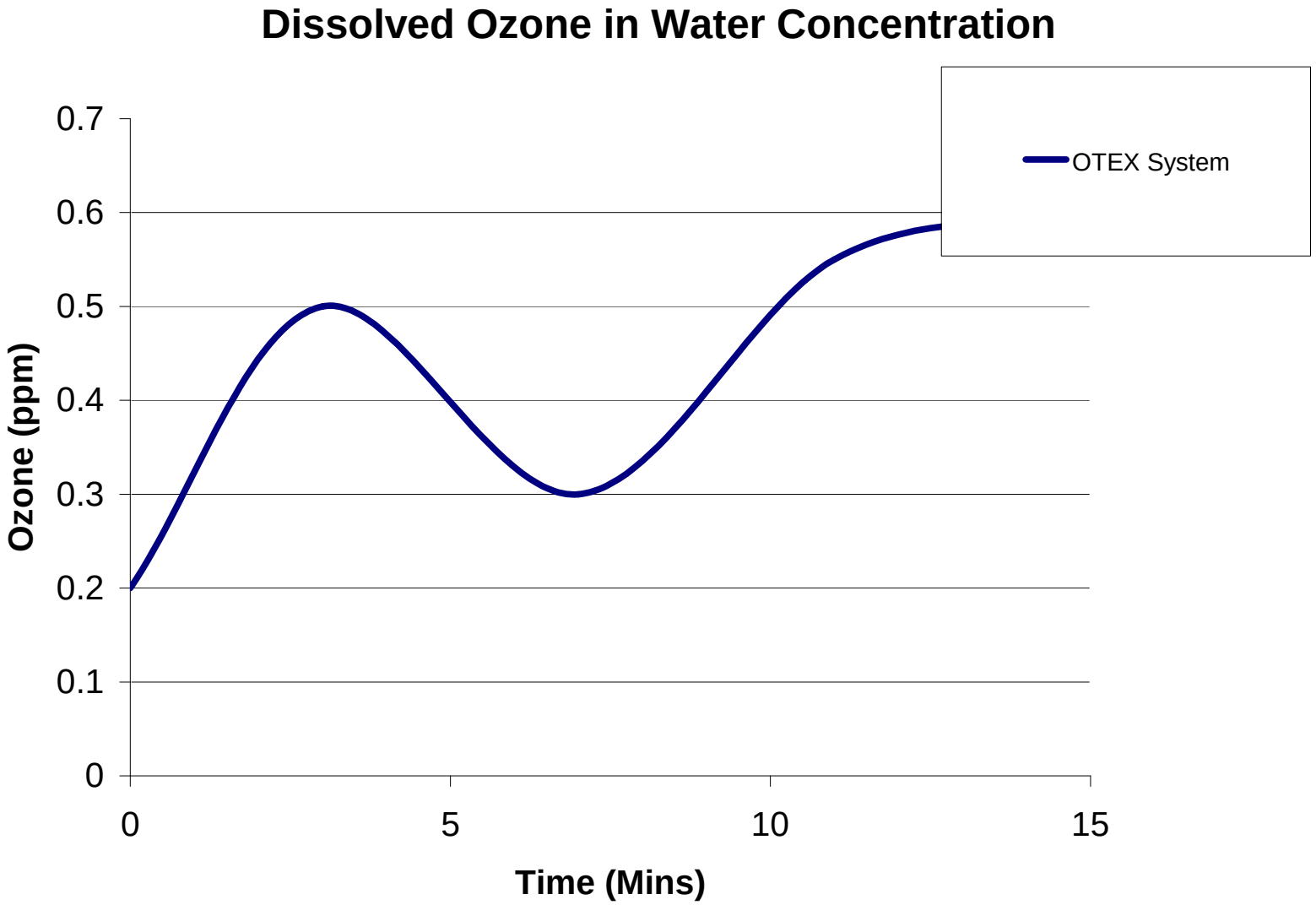
Ozone Concentration	8 Bars
Pressure	5 psi
Flow Rate	3.5cfh

Test samples were taken from the wash drum throughout the wash cycle to determine the concentration of dissolved ozone in the water . This was measured by using the Chemets 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 tabulated below and are expressed in ppm.

4. TEST RESULTS

4.1 Dissolved Ozone Measurements

Time Intervals (Mins)	**JLA's OTEX System Dissolved Ozone (ppm)**
0	**0.2**
3	**0.5**
7	**0.3**
11	**0.55**
15	**0.6**


Dissolved Ozone in Water Concentration
OTEX System
Ozone (ppm)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
5
10
15
Time (Mins)

4.2 Bacteriological Test Results

Trial: CONTROL
No Ozone – Ambient water temperature

	Time Interval (mins)				
	0	3	7	11	15
JLA Lab Ref No:	A1	A2	A3	A4	A5
Micro-organism	*cfu/ml*	*cfu/ml*	*cfu/ml*	*cfu/ml*	*cfu/ml*
Staphylococcus aureus	*1.3E+08*	*1.2E+08*	*8.9E+07*	*8.1E+07*	*7.3E+07*
Pseudomonas aeruginosa	*3.1E+09*	*2.4E+09*	*7.4E+08*	*4.3E+08*	*3.5E+08*
Candida Albicans	*3.1E+08*	*2.2E+08*	*1.5E+08*	*1.4E+08*	*9.6E+07*
Escherichia coli	*5.2E+08*	*3.6E+08*	*1.2E+08*	*9.9E+07*	*8.7E+07*
Streptococcus faecalis	*5.0E+08*	*3.2E+08*	*2.5E+08*	*1.6E+08*	*1.1E+08*
Aspergillus niger	*3.1E+08*	*2.5E+08*	*2.4E+08*	*1.3E+08*	*9.9E+07*
Clostridium difficile	*4.2E+08*	*2.6E+08*	*9.9E+07*	*7.0E+07*	*4.1E+07*
Clostridium perfringens	*9.2E+08*	*6.7E+08*	*6.5E+08*	*6.0E+08*	*5.4E+08*
Campylobacter jejuni	*6.0E+08*	*5.4E+08*	*2.5E+08*	*2.1E+08*	*1.9E+08*
Aeromonas mixed species	*8.2E+08*	*8.0E+08*	*4.1E+08*	*1.7E+08*	*1.1E+08*
Actinobacter sps	*4.3E+08*	*1.9E+08*	*5.9E+07*	*3.7E+07*	*3.5E+07*
Lactobacilli sps	*3.9E+08*	*2.2E+08*	*1.7E+08*	*1.4E+08*	*7.1E+07*
Virus particle	*Particles/ml*	*Particles/ml*	*Particles/ml*	*Particles/ml*	*Particles/ml*
Lambda phage	*3.8E+24*	*2.1E+23*	*1.2E+22*	*1.3E+21*	*3.3E+19*
FCoVA	*2.6E+24*	*3.8E+23*	*2.1E+22*	*7.7E+20*	*3.1E+19*
Saccharomyces virus ScV-L-BC	*3.1E+23*	*9.4E+21*	*1.7E+21*	*1.2E+20*	*3.3E+20*
Vibrio phage fs1	*2.6E+28*	*7.4E+26*	*1.8E+25*	*1.3E+24*	*1.3E+23*

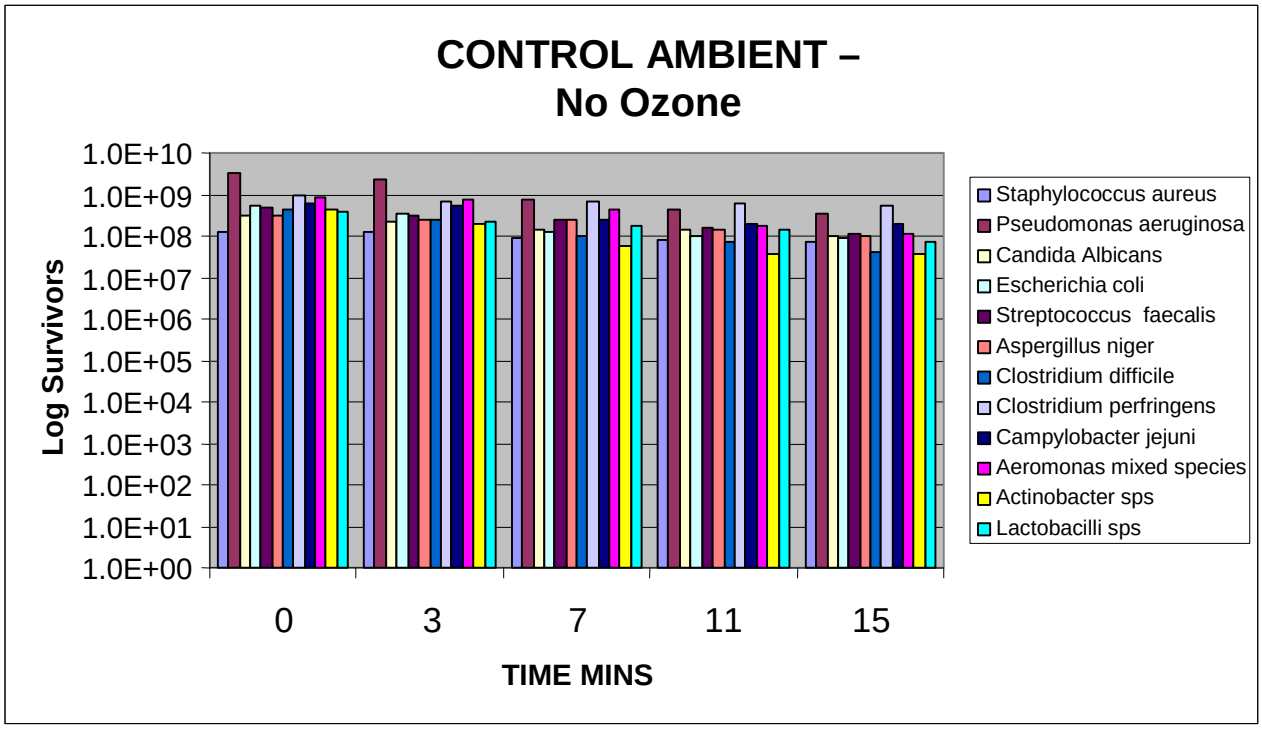
Trial: THERMAL 75°C Water Temperature No Ozone

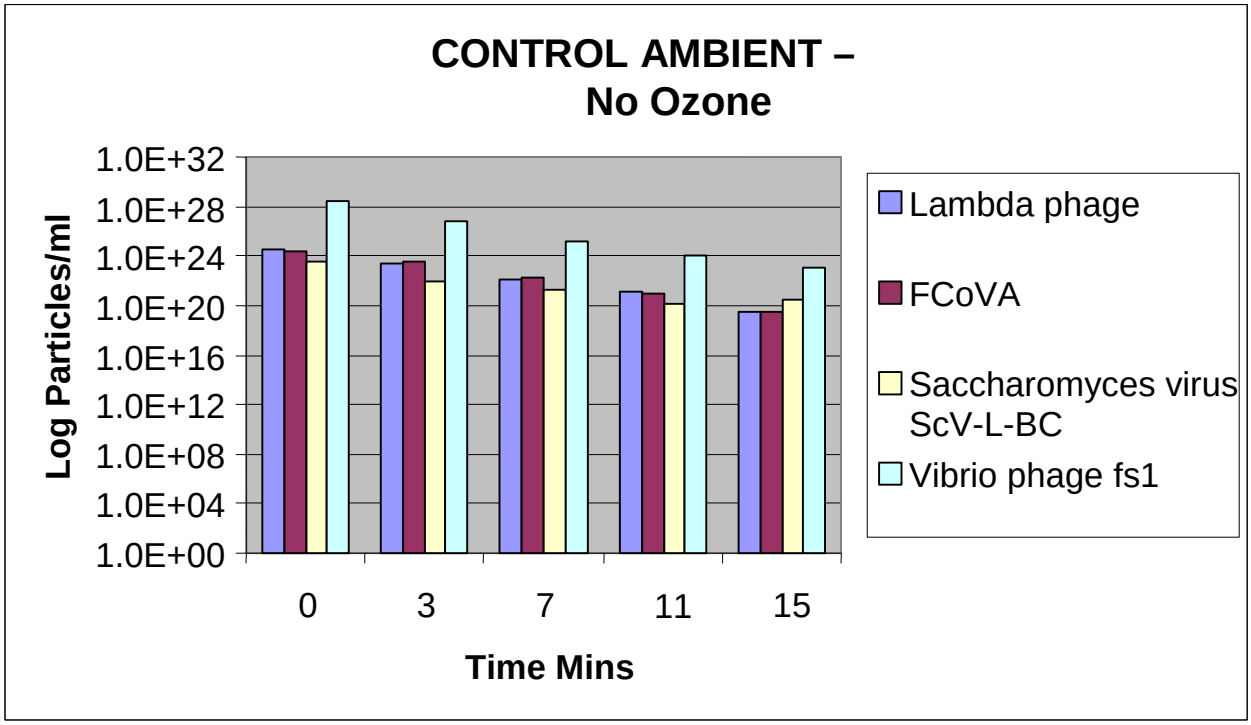
	Time Interval (mins)				
	0	3	7	11	15
JLA Lab Ref No:	C1	C2	C3	C4	C5
Micro-organism	***cfu/ml***	***cfu/ml***	***cfu/ml***	***cfu/ml***	***cfu/ml***
Staphylococcus aureus	**1.3E+08**	**0.0E+01**	**0.0E+01**	**0.0E+01**	**0.0E+01**
Pseudomonas aeruginosa	**3.1E+09**	**0.0E+01**	**0.0E+01**	**0.0E+01**	**0.0E+01**
Candida Albicans	**3.1E+08**	**0.0E+01**	**0.0E+01**	**0.0E+01**	**0.0E+01**
Escherichia coli	**5.2E+08**	**0.0E+01**	**0.0E+01**	**0.0E+01**	**0.0E+01**
Streptococcus faecalis	**5.0E+08**	**4.0E+07**	**8.2E+06**	**1.7E+04**	**3.0E+04**
Aspergillus niger	**3.1E+08**	**0.0E+01**	**0.0E+01**	**0.0E+01**	**0.0E+01**
Clostridium difficile	**4.2E+08**	**4.2E+06**	**6.1E+06**	**7.1E+05**	**8.2E+05**
Clostridium perfringens	**9.2E+08**	**7.2E+05**	**3.0E+04**	**8.6E+04**	**1.3E+05**
Campylobacter jejuni	**6.0E+08**	**0.0E+01**	**0.0E+01**	**0.0E+01**	**0.0E+01**
Aeromonas mixed species	**8.2E+08**	**0.0E+01**	**0.0E+01**	**0.0E+01**	**0.0E+01**
Actinobacter sps	**4.3E+08**	**0.0E+01**	**0.0E+01**	**0.0E+01**	**0.0E+01**
Lactobacilli sps	**3.9E+08**	**0.0E+01**	**0.0E+01**	**0.0E+01**	**0.0E+01**
Virus particle	***Particles/ml***	***Particles/ml***	***Particles/ml***	***Particles/ml***	***Particles/ml***
Lambda phage	**3.8E+24**	**5.1E+12**	**0.0E+01**	**0.0E+01**	**0.0E+01**
FCoVA	**2.6E+24**	**4.1E+07**	**8.0E+02**	**0.0E+01**	**0.0E+01**
Saccharomyces virus ScV-L-BC	**3.1E+23**	**9.0E+12**	**3.1E+06**	**0.0E+01**	**0.0E+01**
Vibrio phage fs1	**2.6E+28**	**5.3E+12**	**0.0E+01**	**0.0E+01**	**0.0E+01**

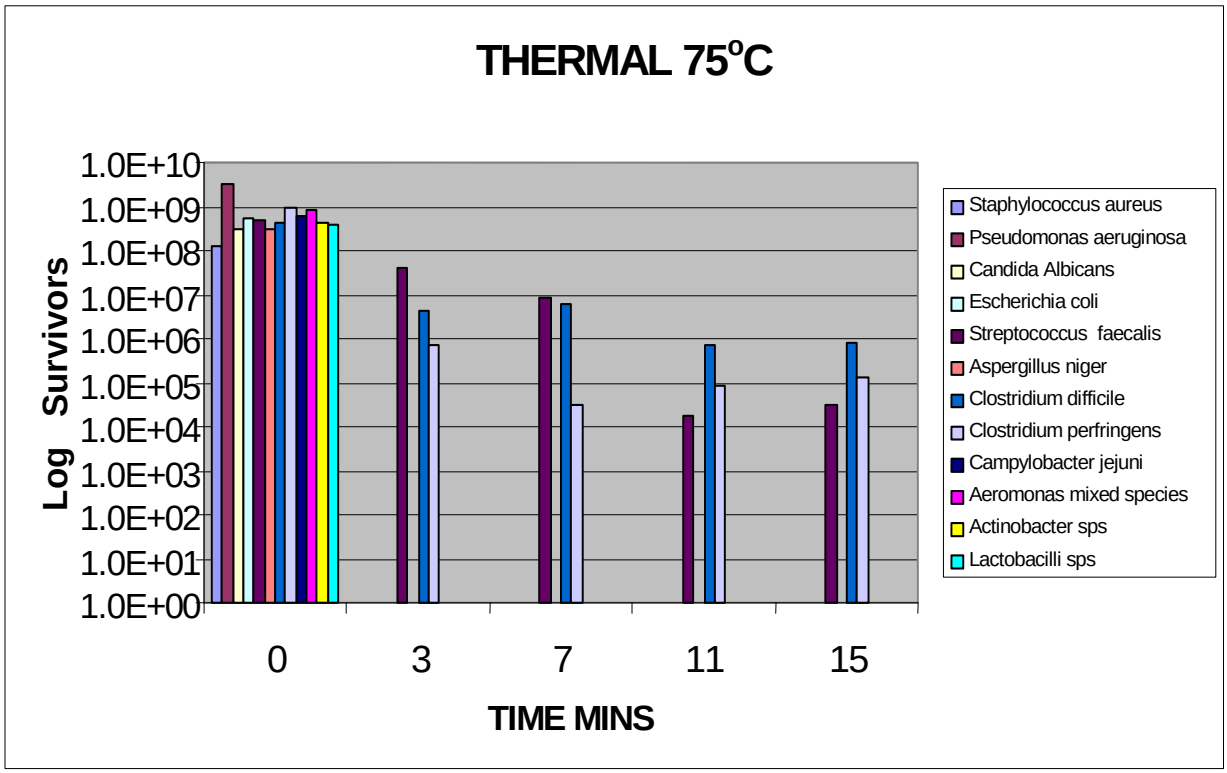
Trial: OTEX Ambient Temperature Water
Mean of two trials

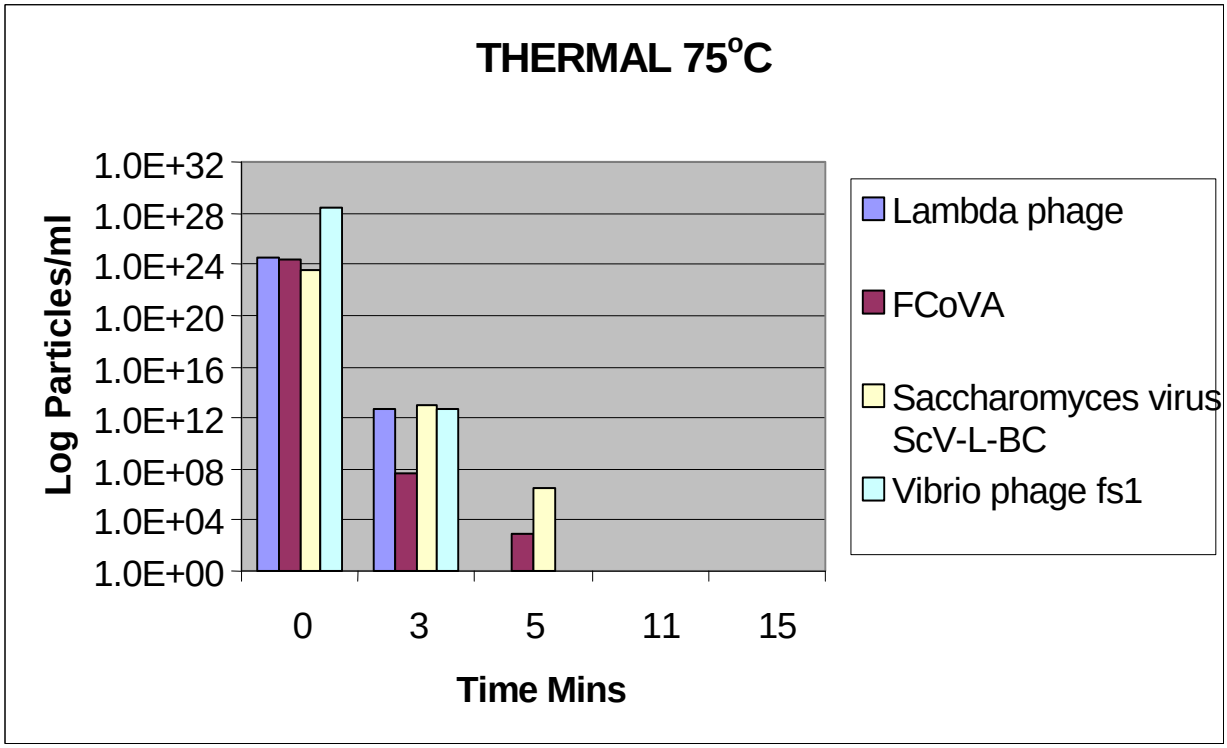
	Time Intervals (mins)				
	0	3	7	11	15
JLA Lab Ref No:	B1	B2	B3	B4	B4
Micro-organism	*cfu/ml*	*cfu/ml*	*cfu/ml*	*cfu/ml*	*cfu/ml*
Staphylococcus aureus	1.0E+08	0.0E+00	0.0E+00	0.0E+00	0.0E+00
Pseudomonas aeruginosa	2.3E+08	0.0E+00	0.0E+00	0.0E+00	0.0E+00
Candida Albicans	4.2E+08	0.0E+00	0.0E+00	0.0E+00	0.0E+00
Escherichia coli	1.7E+08	0.0E+00	0.0E+00	0.0E+00	0.0E+00
Streptococcus faecalis	2.6E+08	0.0E+00	0.0E+00	0.0E+00	0.0E+00
Aspergillus niger	3.0E+08	0.0E+00	0.0E+00	0.0E+00	0.0E+00
Clostridium difficile	3.6E+08	0.0E+00	0.0E+00	0.0E+00	0.0E+00
Clostridium perfringens	4.2E+08	0.0E+00	0.0E+00	0.0E+00	0.0E+00
Campylobacter jejuni	1.2E+08	0.0E+00	0.0E+00	0.0E+00	0.0E+00
Aeromonas mixed species	4.6E+08	0.0E+00	0.0E+00	0.0E+00	0.0E+00
Actinobacter sps	3.9E+08	0.0E+00	0.0E+00	0.0E+00	0.0E+00
Lactobacilli sps	4.6E+08	0.0E+00	0.0E+00	0.0E+00	0.0E+00
Virus particle	*Particles/ml*	*Particles/ml*	*Particles/ml*	*Particles/ml*	*Particles/ml*
Lambda phage	3.8E+24	6.2E+12	0.0E+01	0.0E+01	0.0E+01
FCoVA	2.6E+24	9.1E+07	0.0E+01	0.0E+01	0.0E+01
Saccharomyces virus ScV-L-BC	3.1E+23	4.3E+16	0.0E+01	0.0E+01	0.0E+01
Vibrio phage fs1	2.6E+28	6.0E+08	0.0E+01	0.0E+01	0.0E+01

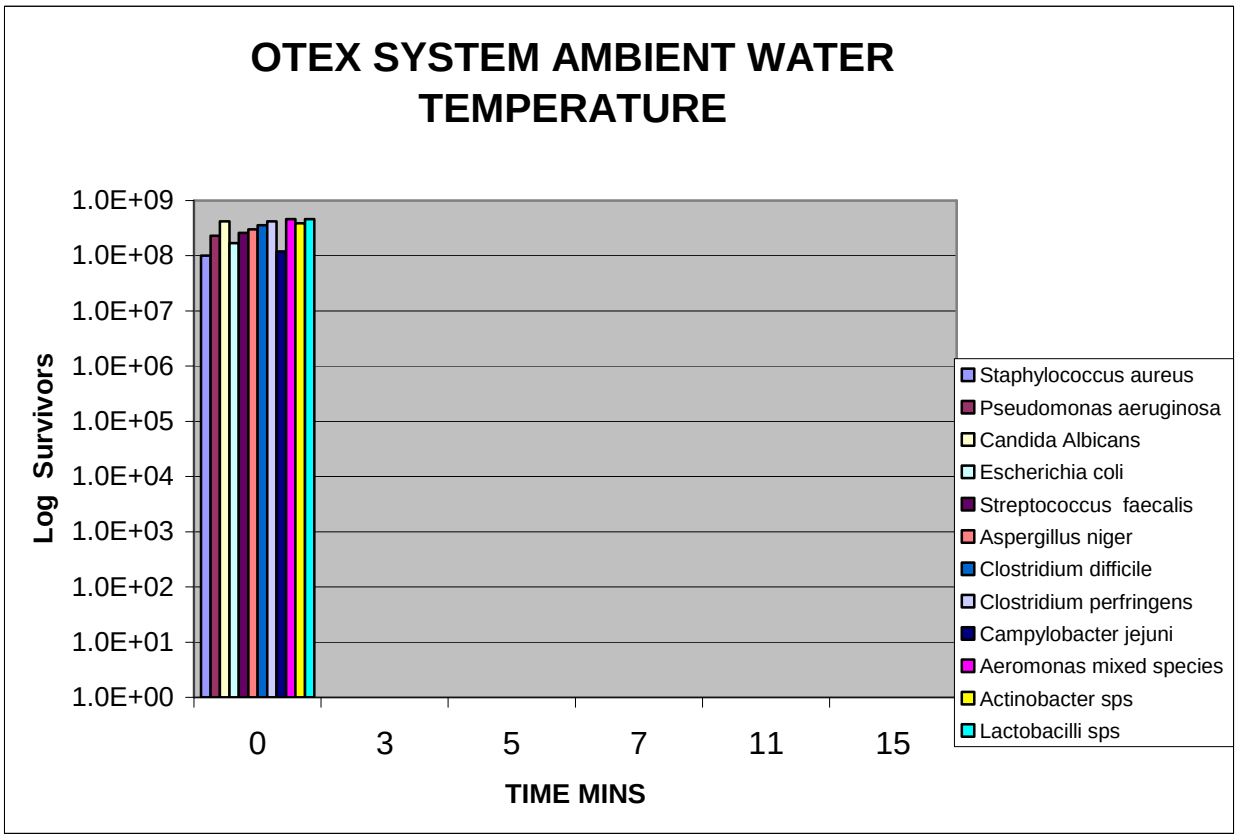
Graphical Representation of Test Results:

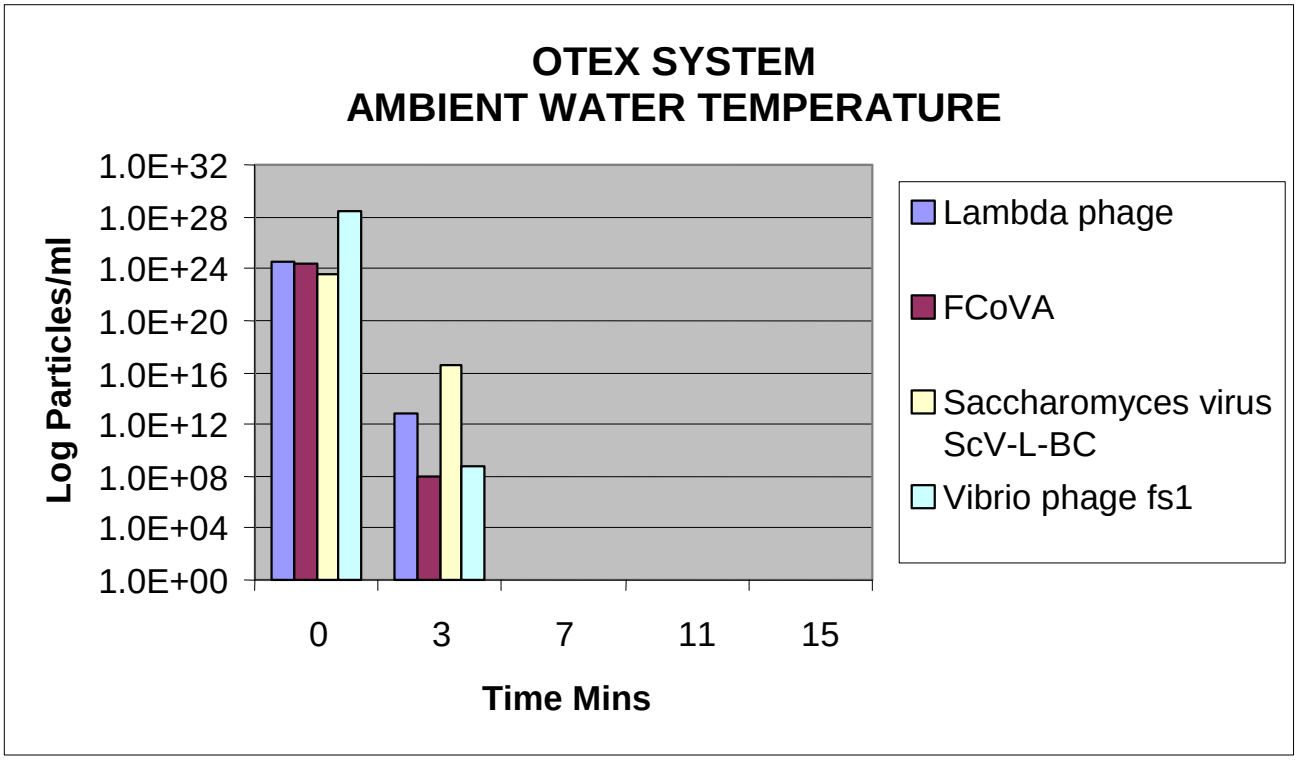





THERMAL 75°C
Log Survivors
1.0E+10
1.0E+09
1.0E+08
1.0E+07
1.0E+06
1.0E+05
1.0E+04
1.0E+03
1.0E+02
1.0E+01
1.0E+00
0
3
7
11
15
TIME MINS
Staphylococcus aureus
Pseudomonas aeruginosa
Candida Albicans
Escherichia coli
Streptococcus faecalis
Aspergillus niger
Clostridium difficile
Clostridium perfringens
Campylobacter jejuni
Aeromonas mixed species
Actinobacter sps
Lactobacilli sps


THERMAL 75°C
Log Particles/ml
1.0E+32
1.0E+28
1.0E+24
1.0E+20
1.0E+16
1.0E+12
1.0E+08
1.0E+04
1.0E+00
0
3
5
11
15
Time Mins
Lambda phage
FCoVA
Saccharomyces virus ScV-L-BC
Vibrio phage fs1


OTEX SYSTEM AMBIENT WATER TEMPERATURE
Log Survivors
1.0E+09
1.0E+08
1.0E+07
1.0E+06
1.0E+05
1.0E+04
1.0E+03
1.0E+02
1.0E+01
1.0E+00
0
3
5
7
11
15
TIME MINS
Staphylococcus aureus
Pseudomonas aeruginosa
Candida Albicans
Escherichia coli
Streptococcus faecalis
Aspergillus niger
Clostridium difficile
Clostridium perfringens
Campylobacter jejuni
Aeromonas mixed species
Actinobacter sps
Lactobacilli sps


OTEX SYSTEM
AMBIENT WATER TEMPERATURE
Log Particles/ml
1.0E+32
1.0E+28
1.0E+24
1.0E+20
1.0E+16
1.0E+12
1.0E+08
1.0E+04
1.0E+00
0
3
7
11
15
Time Mins
Lambda phage
FCoVA
Saccharomyces virus ScV-L-BC
Vibrio phage fs1

6 CONCLUSION

A solution containing bacteria and viruses was introduced into a commercial laundry machine under controlled laboratory conditions to determine the survival rate of the organisms in contact with ozonated water. Tests carried out using both ambient and hot water i.e. 75°C were devised to provide comparative data on current laundry practices

The results produced by independent microbiologists Microsearch Laboratories Ltd, confirm that the use of ozone within the wash process has a significant affect on the reduction of bacterial, fungicidal and viral activity .

APPENDIX A:
Structural and Genomic Information on Viruses

Table below details structural and genomic information relating to the virus particles employed during this series of experiments.

Virus	Nucleic acid	Family	Genome Data
E.coli T4 Phage	ds DNA	Myoviridae (T4 like phages)	Genomes have a Mr of about 120 10^6 (169 kbp), corresponding to 48% of particle weight, inasmuch as known contain 5-hydroxymethylcytosine (HMC) instead of thymine and are glycosylated, have a G+C content of 35%, and are circularly permuted and terminally redundant.
$FCoV^A$	"+" ss RNA	Nidovirales (genus corona virus)	The Corona virus genome is an infectious, linear, positive-sense, polyadenylated and, at least for arteri- and coronaviruses, 5 capped ssRNA molecule. The size Coronavirus is 20 to 25 kb . The coronavirus genome is the largest known non-fragmented viral RNA genome.
Saccaharomyces virus ScV-L-BC	ds RNA	Totiviridae	Virions contain a single linear molecule of uncapped dsRNA (4.6–6.7 kbp in size). The positive strand has two large overlapping ORFs; the length of the overlap varies from 16 to 130 nts. The first ORF encodes the viral major capsid protein with a predicted size of 76–81 103. In the case of ScV-L-A, the two reading frames together encode, via translational frameshift, the putative RNA-dependent RNA polymerase as a fusion protein (analogous to gag-pol fusion proteins of the retroviruses) with a predicted Mr of 170 103.
Vibrio phage fs1	ss DNA	Inovirdiae	Virions contain one molecule of infectious, circular, positive sense ssDNA. Inovirus genomes range from 6 kb to 9 kb.

$FCoV^A$ attenuated non transmissible variant