

A Technical report on the Anti-microbial
performance of the Bactokill air sterilisation
device in the treatment of airborne Bacteria,
Fungi and Viruses

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1.0 Introduction ; My name is R.D.O'Connor, I hold B.Sc.(Hons) degree in applied environmental microbiology and I am a chartered Biologist (Ci.Biol.). I currently work as a practising microbiologist and C.E.O. of a UKAS accredited, DEFRA (formerly MAFF) authorised contract laboratory, authorised of handling Class II pathogens.

1.1 My colleagues and I were invited to evaluate the Bactokill device by . This report documents the findings obtained during a series of studies designed to demonstrate the anti-microbial performance of the Bactokill air sterilisation unit.

1.2 The scope of this report includes ;

- i) Documentation of the Bactokill device in the *in vitro* inactivation of a range of bacterial species and fungi
- ii) Documentation of the performance of the Bactokill device in the *in vitro* inactivation of a range of viral particles.
- iii) Documentation of two field trials during which the Bactokill device was employed to process atmospheres known to historically exhibit unacceptable and problematic airborne microbial loading.

2.0 Microbial Protocols ;

2.1 Protocols employed for the measurement of the performance of Bactokill device in the *in vitro* inactivation of a range of Bacterial fungal forms.

a) In vitro kill rate trials

The demonstration of antibacterial and antifungal efficiency *in vitro* was by achieved repeated nebulisation of cultures via the intake section of the Bactokill device. Recovery was achieved by measurement of the expelled atmosphere which was collected in a vented chamber enclosing the output section of the device. In total 30 measurements were made for each organism at each of a variety of flow rates. Recovery of the atmosphere was achieved by aspiration of known volumes of atmosphere into a physiological saline collection trap, after which enumeration was achieved by standard plate counts.

Control trials were also performed to measure lethality or non recoverability due to physical factors other than by the intended means of sanitisation. These trials were conducted as described above but with the UVc source disconnected. Lethality due to none UVc effects was negligible

2.2 Protocols employed for the measurement of the performance of Bactokill device in the *in vitro* inactivation of a range of viral particles.

Figure 1 below details structural and genomic information relating to the virus particles employed during this series of experiments.

Figure 1

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.
wFCoV ^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.
Saccharomyces 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	Inoviridae	Virions contain one molecule of infectious, circular, positive sense ssDNA. Inovirus genomes range from 6 kb to 9 kb.
<i>FCoV^A attenuated non transmissible variant</i>			

2.4 For this trial all viral particles were cultured in host cell lines. In the case of T4 phage, ScV-L-BC and fs1, each virus was obtained by enrichment from continuous culture vessels. FCoV was obtained after culture in a continuous epithelial cell line.

Primary cultures were subject to ultra sonic fractionation, followed by filtration and centrifugation. In this manner Infective dispersions intended for aspiration were obtained in Phosphate buffered saline. Fresh viral dispersion were created and calibrated for each period of measurement. Nebulised cultures were recovered as described above and were examined quantitative recovery by the processes described in figure 2 below.

Figure 2 summarises the techniques employed to enumerate viral suspensions from any source during this trial.

Fig.2

Virus	Mode of Enumeration
E.coli T4 Phage	Plague formation
FCoV ^A	Real time PCR
Saccharomyces virus ScV-L-BC	Plague formation /cytopathy
Vibrio phage fs1	Plague formation /cytopathy

FCoV^A attenuated non transmissible variant

4.0 Field trial protocols ;

4.1 In all two field trials are documented. These comprise measurements of the efficiency of the Bactokill device in the sanitisation of atmospheric environments known to have significant levels of airborne microbial contamination.

The first trial involved studies in laundry storage (28.7 M³) area which exhibited defined problems with airborne bacteria and moulds. During this trial air quality was monitored every 4 hours for a three week period with no UVc treatment. During a second three week monitoring period the effect of UVc treatment due to the Bactokill device was determined using the same sampling plan. Recovery of isolates was by impaction onto solid agar plates using a Cassella sampling device.

A second field trial was conducted at an industrial laboratory which had a know mould (primarily Neurospora species) problem occurring in a sample storage incubator. In this trial we conducted daily control measurements over a 30 day period followed by the same protocol with Bactokill device in operation.

Table 1 Bactokill single pass Kill rates at differing flow rates with a range of organisms showing mean percentage survivors for 30 nebulisations

Organism	Class of organism	Challenge level cfu/m3	Flow setting 14	Flow setting 16	Flow setting 18.5	Flow setting 21.5	Flow setting 24.5
Aeromonas aerogens	Bacterium	2.1E+07	> 99.999	> 99.999	98.4	92.6	85.4
Bacillus cereus	Bacterium	3.2E+07	> 99.999	99.3	94.3	83.2	67.3
Bacillus globigii	Bacterium	4.3E+07	> 99.999	98.7	94.2	81.7	68.3
Bacillus megaterium	Bacterium	4.6E+07	> 99.999	99.6	95	80.3	51.6
Bacillus subtilis	Bacterium	2.9E+07	> 99.999	99.7	96.2	79.3	67.2
Ecoli O157 H:7	Bacterium	1.3E+05	> 99.999	> 99.999	> 99.999	91.1	87.3
Enterobacter agglomerans	Bacterium	6.2E+07	> 99.999	> 99.999	> 99.999	93.3	87.2
Enterobacter gergoviae	Bacterium	3.6E+07	> 99.999	> 99.999	> 99.999	96.1	79.5
Enterobacter sakazakii	Bacterium	3.6E+07	> 99.999	> 99.999	99.1	97.2	68.9
Escherichia coli	Bacterium	2.9E+07	> 99.999	> 99.999	> 99.999	98.3	83.4
Micrococcus luteus	Bacterium	4.3E+07	> 99.999	> 99.999	> 99.999	98.4	61.5
Pseudomonas aeruginosa	Bacterium	3.2E+07	> 99.999	> 99.999	> 99.999	96.3	73.6
Pseudomonas putida	Bacterium	1.4E+07	> 99.999	> 99.999	99.2	90.2	68.2
Salmonella typhi murium	Bacterium	2.8E+07	> 99.999	> 99.999	> 99.999	91.6	91.3
Serratia marcescens	Bacterium	6.3E+07	> 99.999	> 99.999	99.4	89.7	69.1
Staphylococcus aureus MSRA	Bacterium	3.2E+07	> 99.999	98.6	97.6	90.4	87.3
Staphylococcus aureus oxford	Bacterium	3.8E+07	> 99.999	98.7	96.5	91	87.6
Staphylococcus epidermidis	Bacterium	4.1E+07	> 99.999	97.3	96.4	90.4	82.6
Streptococcus pyogenes	Bacterium	2.9E+07	> 99.999	97.5	93.6	87.5	67.8
Streptococcus faecalis	Bacterium	3.8E+07	> 99.999	95.3	90.1	82.9	69.3
Saccharomyces cerevisiae	Yeast	3.6E+07	> 99.999	97.2	83.6	71.4	51.7
Saccharomyces bailli	Yeast	3.0E+07	> 99.999	96.4	87.8	75.3	56.4
Aspergillus niger	Mould	2.9E+07	> 99.999	96.3	82.5	56.3	34.8
Aspergillus flavus	Mould	1.3E+07	> 99.999	97.2	89.3	41.2	21.5
A. niger	Mould	8.2E+06	> 99.999	98.4	76	51.4	33.8
A. flavus	Mould	7.1E+07	> 99.999	96.3	85.1	52.3	28.1
F. poea	Mould	5.0E+07	> 99.999	97.2	87.4	47.5	23.7
P. digitatum	Mould	3.2E+07	> 99.999	98.6	83.4	38.1	28.1
F. gramineum	Mould	2.9E+07	> 99.999	99.3	84.6	46.2	26.9

Table 2 Bactokill Kill rates at differing flow rates with a range of Virus particles showing mean percentage survivors for 30 nebulisations

Organism	Class of Viron	Challenge level cfu/m3	Flow setting 14	Flow setting 16	Flow setting 18.5	Flow setting 21.5	Flow setting 21.5
T4 Phage	ds DNA	5.1E+12	> 99.999	> 99.999	> 99.999	94.7	90.3
FCoVA	"+" ss RNA	6.2E+12	> 99.999	> 99.999	> 99.999	93.6	91.6
ScV-L-BC	ds RNA	7.9E+12	> 99.999	> 99.999	> 99.999	93.2	89.2
fs1 phage	ss DNA	6.0E+12	> 99.999	> 99.999	> 99.999	96.1	91.8

Table 3 Mean Microbial Levels over a 24 hour period in a Laundry Storage room (28.7 M³) without continuous operation of the Bactokill device over a three week period

Flow setting 18.5 IL-1 sec

TIME	Oxidase Pos Gram negative isolates L ¹ /air	Oxidase Pos Gram negative isolates L ¹ /air	Yeasts Moulds L ¹ /air	Gram Positive isolates L ¹ /air
6:00 AM	3.3E+02	9.0E+02	3.1E+03	1.4E+03
10:00 AM	1.9E+03	1.2E+03	8.7E+03	3.2E+03
2:00 PM	3.1E+04	2.8E+03	2.1E+04	2.6E+04
6:00 PM	3.6E+04	1.9E+03	5.3E+04	1.7E+04
10:00 PM	1.7E+03	7.4E+02	1.2E+04	9.1E+03
2.00 AM	8.3E+02	8.3E+02	9.1E+03	5.2E+03
4.00 AM	4.2E+02	9.3E+02	5.7E+03	1.1E+03

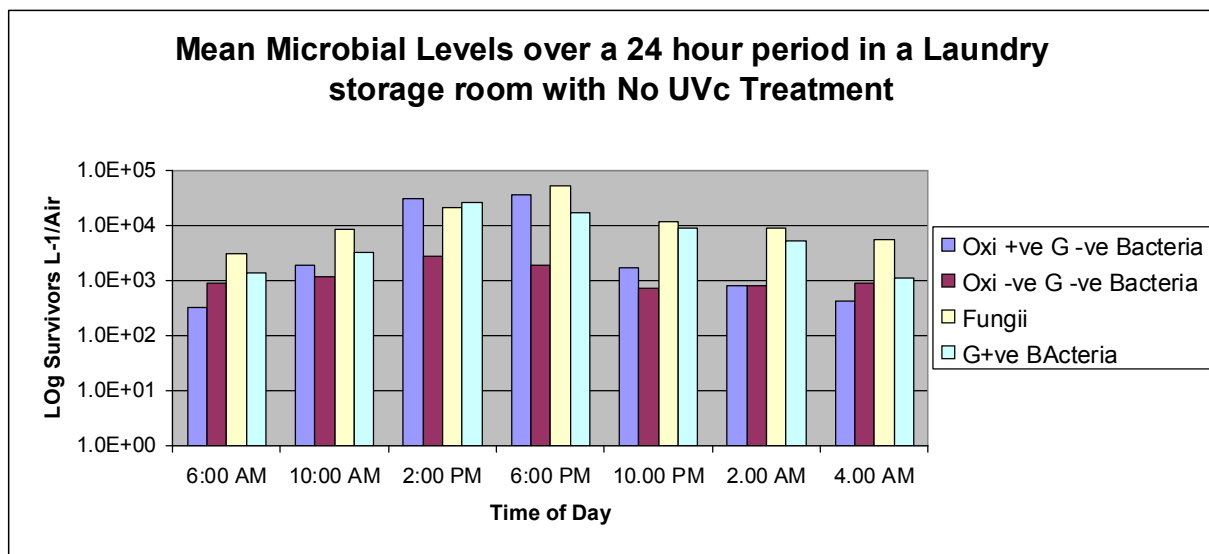


Table 4 Mean Microbial Levels over a 24 hour period in a Laundry Storage room (28.7 M³) WITH continuous operation of the Bactokill device over a three week period

Flow setting 18.5 L-1 sec

TIME	Oxidase Pos Gram negative isolates L ¹ /air	Oxidase Pos Gram negative isolates L ¹ /air	Yeasts Moulds L ¹ /air	Gram Positive isolates L ¹ /air
6:00 AM	3.0E+01	2.8E+01	5.3E+02	3.3E+02
10:00 AM	8.0E+01	2.0E+01	9.8E+02	3.4E+02
2:00 PM	3.0E+02	1.5E+02	9.4E+02	8.7E+02
6:00 PM	4.3E+02	3.9E+02	7.1E+03	5.4E+02
10:00 PM	1.8E+02	1.2E+02	3.6E+03	3.1E+02
2:00 AM	3.4E+01	9.4E+01	1.6E+02	9.3E+01
4:00 AM	1.7E+01	1.2E+01	2.1E+02	1.3E+02
MEAN % REDUCTION	96.3	91.5	88.3	91.7

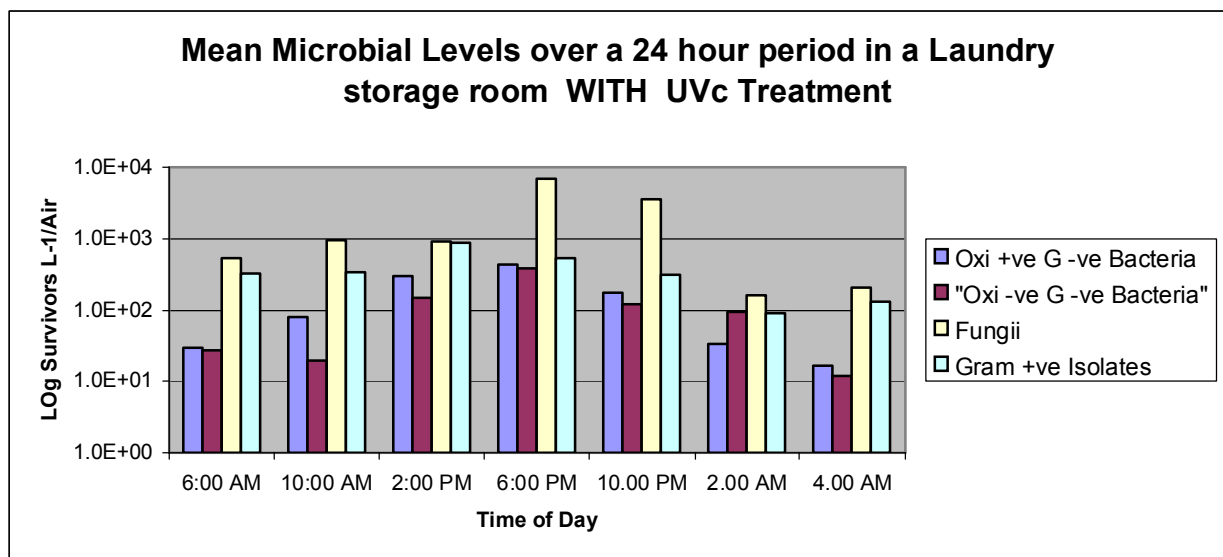
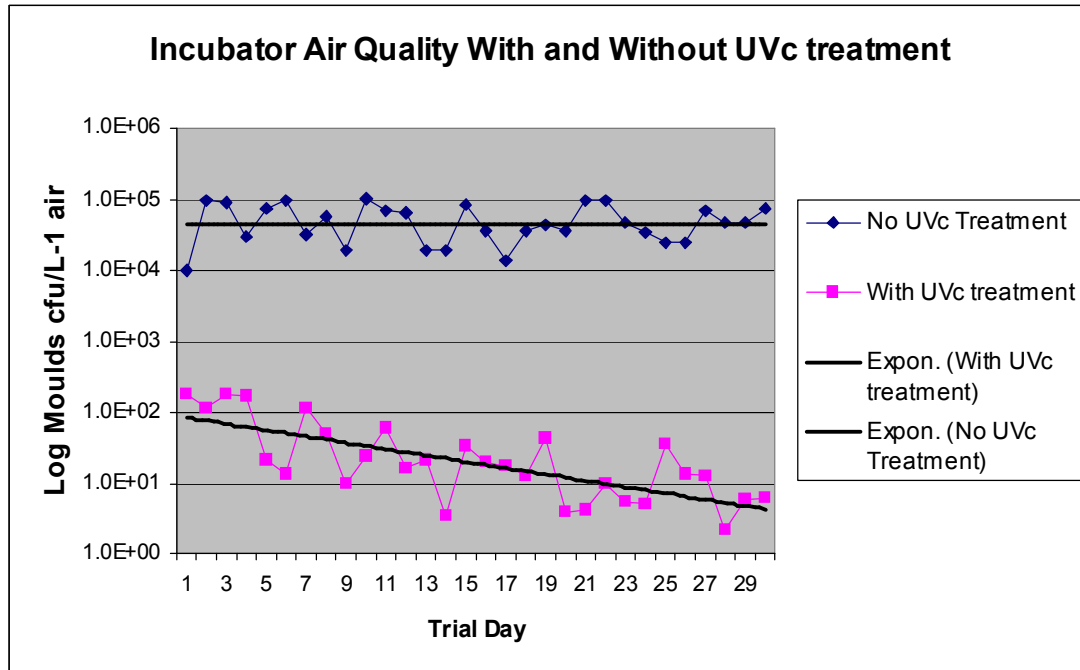


Table 5 Mould levels recovered from an incubator (19.4 m³) atmosphere over 30 days period with and without Bactokill Uvc treatment.

Flow setting 18.5 l-1/second

Day	NO Uvc treatment Mould isolates L ¹ /air	NO Uvc treatment Mould isolates L ¹ /air
1	1.0E+04	1.8E+02
2	1.0E+05	1.2E+02
3	9.3E+04	1.8E+02
4	3.1E+04	1.7E+02
5	7.3E+04	2.2E+01
6	9.9E+04	1.4E+01
7	3.2E+04	1.1E+02
8	5.9E+04	5.0E+01
9	2.0E+04	9.8E+00
10	1.0E+05	2.4E+01
11	7.2E+04	6.0E+01
12	6.4E+04	1.7E+01
13	1.9E+04	2.1E+01
14	1.9E+04	3.5E+00
15	8.3E+04	3.3E+01
16	3.6E+04	1.9E+01
17	1.4E+04	1.7E+01
18	3.7E+04	1.3E+01
19	4.5E+04	4.2E+01
20	3.7E+04	3.9E+00
21	9.6E+04	4.1E+00
22	9.9E+04	9.5E+00
23	4.7E+04	5.3E+00
24	3.5E+04	5.0E+00
25	2.5E+04	3.6E+01
26	2.5E+04	1.3E+01
27	6.9E+04	1.2E+01
28	4.7E+04	2.1E+00
29	4.8E+04	5.7E+00
30	7.5E+04	6.3E+00
mean	5.4E+04	4.0E+01
	Mean % reduction	99.9
	Mean Log reduction	3.1



Discussion :-

The Bactokill device according to specification achieves decontamination of airborne microbial populations by UVc treatments. This series of trials has sought to validate this claim.

UV light has been studied since the 1930's and has been shown, under proper conditions of exposure and intensity to exhibit lethal anti-microbial effects against Bacteria, fungi and virus particles (viii, ix, x).

It has been shown that UVc (xi) inactivates pathogens according to the standard decay equation $S = \exp(-kIt)$. S represents the fraction of the original population, in this equation which survive exposure at time t, and I represents the UVc intensity. The rate constant k has been experimentally determined for a wide range of bacteria, spores, fungi and viruses.

In the literature, the consensus is that generally viruses appear to be more susceptible to UV irradiation than other forms of micro-organisms. A range of derived K values for a range of organisms, in air, are given below.

Organism	$K=cm^2/uj$
Vaccina (2)	$1.53e^{-3}$
Echo virus (3)	$2.17e^{-4}$
Coxsackie virus (2)	$1.11e^{-3}$
Staphylococcus aureus	$3.48e^{-3}$
E.coli	$3.76e^{-3}$

The increased susceptibility demonstrable in many types of virus particles is directly related to the lack of a nucleic acid repair mechanism, lack of shielding whilst in the atmosphere and the impact of UVc irradiation on low gene numbers.

Inactivation of virus particles and other micro-organisms is considered to be due primarily to the mechanism of Pyridine nucleotide dimerisation and at higher energies by chromosome fractionation.

The Bactokill is constructed in a manner which facilitates the passage of contaminated air through a conduit thereby exposing airborne organisms to high doses of UVc light (primarily at 253.7 nanometers with a dosage rate of $> 60,000 \text{ mw/sec/cm}^2$) at flow rate which may be varied between 14 and $24.4 \text{ L}^{-1}/\text{air/minute}$.

Focusing on the in vitro trials involving bacteria and fungi our data indicates that at a flow rate of $14 \text{ L}^{-1}/\text{air/minute}$ the Bactokill device consistently produced $> 99.999\%$ kill with all isolates included in the trial (Table 1). Our data indicates that as flow rate increases there is a predictable drop in kill efficiency with certain categories of organism and in my opinion the degree of lethality achieved is broadly acceptable up to a maximum of $18.5 \text{ L}^{-1}/\text{air/minute}$.

In the case of viral treatment the machine produced a > 99.999 kill rate of for all challenge particles up to and again including a flow rate of $18.5 \text{ L}^{-1}/\text{air/minute}$.

In both field trials the Bactokill device was evaluated in atmospheric environments with known airborne microbial problems.

Of the two environments studied the Laundry storage room was considered to be the most active area in terms of general usage, people hours and formite movement. Our data indicates an average of 37 area visits per day with a approximately 70 % turnover of soiled materials. In the presence of such effects it is predicted that high replacement rates of airborne contaminants would be likely due movement and the flow soiled materials which are transiently stored in that facility. Given such a volatile environment it is impressive that in the case of Bacterial isolates, the Bactokill device produced between a 91-96 % kill level on a continuous basis for Bacteria and an 88.3 % kill level for Yeasts and moulds, at a flow rate of 18.5 L¹/air/minute.

In the case of the industrial incubator trial much higher efficiencies were achieved wherein over a 30 day period, environmental mould levels were reduced by > 99.9% during continuous operation representing an impressive overall 3.1 log cycles of reduction.

Taking an overview the Bactokill device performed impressively during single pass challenge trials with all categories of micro-organism employed in the trials. Further no deficiency in performance were detected during field trials and on this basis the device can be recommended as an important tool in the maintenance of sanitary environments.

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