B R A N D E N B U R G U K L T D .

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Microbiological Report

Evaluation of the anti-viral performance of the Medixair UVc device

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1.0 Introduction

The effectiveness of Brandenburg UK Ltd. UVc air sterilisation unit (Medixair) has previously been successfully evaluated against a wide range of airborne bacteria and fungi, both in the laboratory and in field trials.

Work has been undertaken to demonstrate the potential of the device to reduce airborne viral particles in the environment.

It has already been shown that UVc (Rauth 1965; Setlow 1961) inactivates pathogens according to the standard decay equation.

$$S = \exp(-kIt).$$

Where:

- S = Represents the fraction of the original population, which survive exposure at time t.
- I = UVc intensity.
- k = The rate constant k has been determined experimentally for a range of bacteria, spores, fungi and viruses.

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

Organism	**K=cm^2/μj**
Vaccina (2)	1.53e^{-3}
Echo virus (3)	2.17e^{-4}
Coxsakie virus (2)	1.11e^{-3}

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

"viruses are shown to be more susceptible to UV irradiation than other forms of micro-organisms"

Inactivation of virus particles is considered to be due to the mechanism of pyridine nucleotide dimerisation and at higher energies by chromosome fractionation.

In this study we have we have investigated the performance of the Medixair UVc air sterilisation unit in the inactivation of four common virus groups which cover particles composed of either double strand DNA, single strand DNA, double strand RNA or +single strand RNA.

The work was conducted in specially constructed apparatus consisting of two H.E.P.A. vented chambers (each of 7m^3 volume) interconnected by the Medixair UVc air sterilisation unit. (See figure 1).

The chamber (A) was subject to periodic aspiration with an aerosol containing virus particles. Atmosphere from chamber A was

transferred to chamber B via the Medixair UVc air unit. The numbers of airborne viral particles were monitored in both chambers. By operating these conditions over a period of time, with and without the UVc source in operation, it has been possible to demonstrate the effect of the sanitisation effect of the device on virus particles.

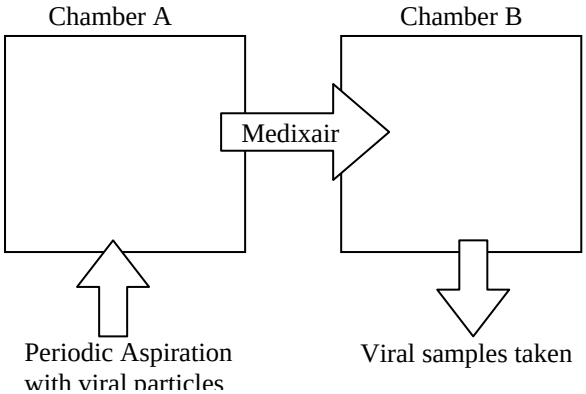


Figure 1. Experimental Apparatus.

In this manner, in contrast to previous work, which involved bacterial strains, we set out to demonstrate the efficiency of the device in respect to a constant flow of viral particles. In this instance, in contrast to previous trials, the airflow was linear.

To clarify, contaminated atmosphere from chamber A was transferred to sampling chamber B by the action of the Medixair fan. Giving the predictions concerning the high degree of viral susceptibility to UVc, it was considered that a re-circulation of particles was not required.

In contrast to previous trials we have introduced the concept of "replacement of challenge" to the experimental design. In this case we have conducted these trials with the delivery of repeated viral dosage to the challenge chamber over a 3.5hour period.

The challenge level was very high, ranging between Log10 and Log 2 viral particles per m^3 of air. The quantity of viral particles was introduced at 30minute intervals during the delivery period.

When interpreting the data it is important to recognise that the experimental design included both evaluation of viral decay, with and without the UVc device operating. The intention was to be able to demonstrate the lethality specifically attributable to the use of the Medixair unit with respect to airborne virus particles.

We have therefore sought to obtain consistent results, which demonstrate repeatability. We consider this approach closely models the type of environmental challenge the Medixair unit was designed to manage.

2.0 The Virus Particles

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

Table A.

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

2.1 Particle Preparation

All particles were cultured in and harvested from 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.

Purified Virions were obtained by ultra-sonic fractionation of the host cells, followed by filtration and centrifugation. Infective dispersions intended for aspiration were prepared in Phosphate buffered saline. Such dispersions were freshly obtained for each period of monitoring.

2.2 Particle Enumeration

Table B summarises the techniques employed for enumerating viable virons sampled from chambers A and B of the test apparatus. Due to the degree of uncertainty associated with viron enumeration, all data was recorded to the nearest integer for all orders of magnitude.

Table B

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

$FCoV^{A}$ attenuated non-transmissible variant

3.0 Experimental Protocol and Sampling

All experiments were conducted in triplicate with appropriate containment and practise.

As described above our intention was to create an elevated level of atmospheric contamination with each type of viral particle in chamber "A". This was achieved by pressurised aspiration of viron particles as an aerosol with a particle size range of between <1 and 25 micron, these were introduced to chamber "A".

Dosing of chamber "A" occurred every 30 minutes for a period of 3.5 hours during each experiment. Air sampling was carried out at time 0 after dosing and 30 minutes thereafter. Sampling continued at thirty minute intervals and although dosing had been finished, air sampling continued for a total time of 4.5 Hours. Phosphate buffered saline was used as the medium for collection of the samples. The viral concentration was determined by the techniques described in table B above.

Air from Chamber "B" which had been passed through the Medixair UVc unit was sampled with the same time intervals, each instance occurring immediately subsequent to sampling of chamber "A".

Performance characteristics for each virus particle were determined by separate experiments.

In order to demonstrate random non-viability, control experiments were conducted by operating with the UVc source inactivated. This data is expressed in Tables 1-4 below, together with the magnitude of inactivation obtained whilst the UVc system was in operation.

The level of log reduction attributable to UVc has been corrected by subtraction of the log kill obtained during trials when the UVc source was not operating.

Results Tables

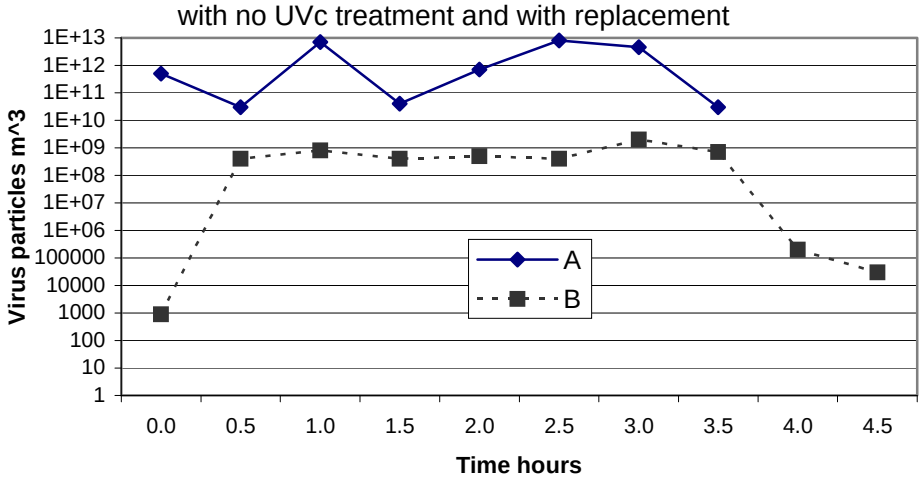
4.0 Results

Table 1 Inactivation of T4 Phage

Aspiration interval Hours	Aspirated virus particles units/m^3	Sampling time post aspiration	Recovered virus particles units/m^3 **UVc on**	Aspirated virus particles units/m^3	Sampling time post aspiration	Recovered virus particles units/m^3 **UVc off**
0.0	2.0E+10	0.0	0.0	5.0E+11	0.0	9.0E+02
0.5	3.0E+10	0.5	3.0E+01	3.0E+10	0.5	4.0E+08
1.0	4.0E+11	1.0	2.0E+02	7.0E+12	1.0	8.0E+08
1.5	7.0E+10	1.5	3.0E+02	4.0E+10	1.5	4.0E+08
2.0	5.0E+12	2.0	1.0E+02	7.0E+11	2.0	5.0E+08
2.5	8.0E+12	2.5	5.0E+02	8.0E+12	2.5	4.0E+08
3.0	4.0E+10	3.0	6.0E+02	4.6E+12	3.0	2.0E+09
3.5	8.0E+10	3.5	7.0E+02	3.0E+10	3.5	7.0E+08
4.0	0.0	4.0	6.0E+00	0.0	4.0	2.0E+05
4.5	0.0	4.5	1.0E+01	0.0	4.5	3.0E+04

UVcOff

Graph 1
Reduction of virus particles over 4.5 hours
with no UVc treatment and with replacement



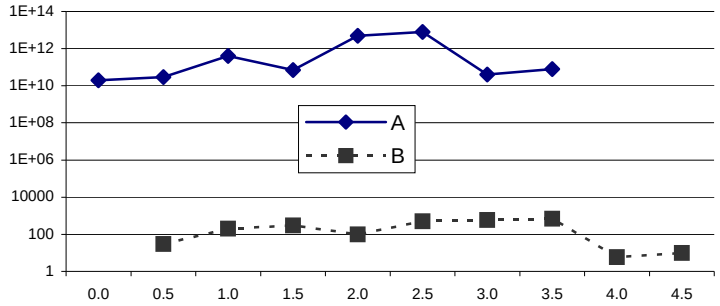
A : Level of Aspirated virus particles per m3 in the pre treatment chamber
B: Level of Aspirated virus particles per m3 in the post treatment chamber UVc off

Summary UVc Off

	Particles	Log
Total particle input over 3,5 hours	2.1E+13	13.3
Total particle recovery over 4,5hrs	5.2E+09	9.7
Depletion - UVc off	2.1E+13	3.6

UVC ON

Graph2
Reduction of Virus particles over 4.5 hours with UVc treatment and with replacement



A : Level of Aspirated virus particles per m^3 in the pre treatment chamber
B : Level of Aspirated virus particles per m^3 in the post treatment chamber UVc on

Summary UVc On

	Particles	Log
Total particle input over 3,5 hours	1.4E+13	13.1
Total particle recovery over 4,5hrs	2.4E+03	3.4
Correction for depletion		3.6
Corrected Depletion UVc on		6.1

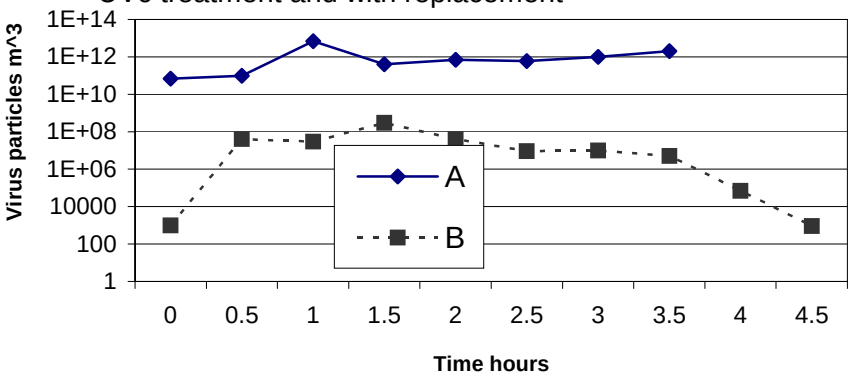
Table 2 Inactivation of Airborne FCoV

Interval Hours	Aspirated virus particles aspiration units/m^3	Sampling time post aspiration	Recovered virus particles units/m^3 **UVc on**	Aspirated virus particles units/m^3	Sampling time post aspiration	Recovered virus particles units/m^3 **UVc off**
0.0	3.0E+10	0.0	0.0	7.0E+10	0.0	1.0E+03
0.5	4.0E+11	0.5	3.0E+02	1.0E+11	0.5	4.0E+07
1.0	4.0E+11	1.0	4.0E+02	7.0E+12	1.0	3.0E+07
1.5	6.0E+10	1.5	7.0E+02	4.0E+11	1.5	3.0E+08
2.0	5.0E+11	2.0	5.0E+02	7.0E+11	2.0	4.0E+07
2.5	4.0E+12	2.5	7.0E+03	6.0E+11	2.5	9.0E+06
3.0	9.0E+11	3.0	4.0E+02	1.0E+12	3.0	1.0E+07
3.5	8.0E+11	3.5	8.0E+02	2.0E+12	3.5	5.0E+06
4.0	0.0	4.0	7.0E+01	0.0	4.0	7.0E+04
4.5	0.0	4.5	0.0E+00	0.0	4.5	9.0E+02

UVc Off

Graph 3

Reduction of Virus particles over 4.5 hours with no UVc treatment and with replacement



A : Level of Aspirated virus particles per m3 in the pre treatment chamber

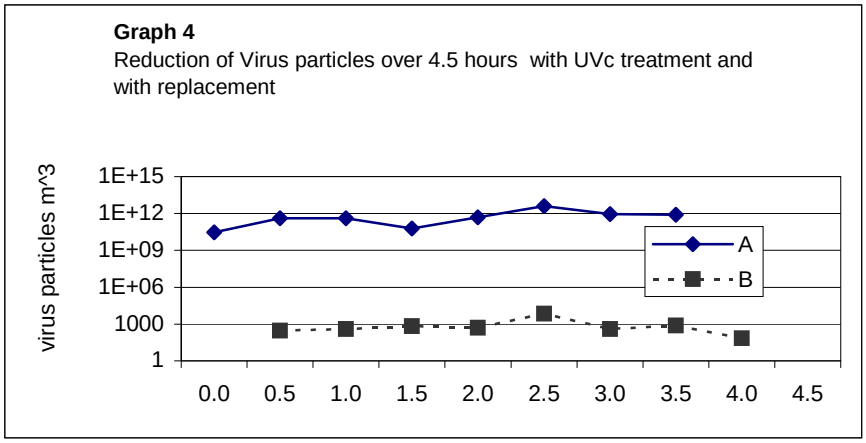
B : Level of Aspirated virus particles per m3 in the post treatment chamber UVc off

Summary UVc Off

	Particles	Log
Total particle input over 3.5 hours	1.2E+13	13.1
Total particle recovery over 4.5	4.3E+08	8.6
Depletion UVc off	1.2E+13	4.4

UVc On

Graph 4
Reduction of Virus particles over 4.5 hours with UVc treatment and with replacement



A : Level of Aspirated virus particles per m^3 in the pre treatment chamber
B : Level of Aspirated virus particles per m^3 in the post treatment chamber UVc on

Summary UVc On

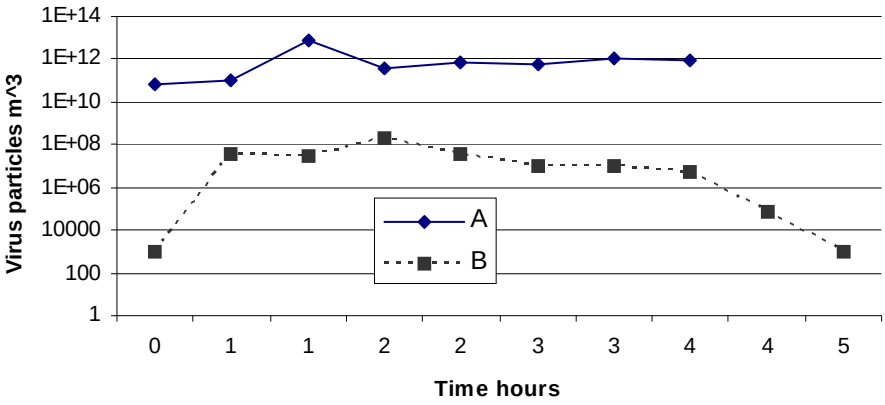
	Particles	Log
Total particle input over 3.5 hours	7.1E+12	12.9
Total particle recovery over 4.5	1.0E+04	4.0
Correecti for Depletion		4.4
Corrected Depletion UVc on		4.4

Table 3 Inactivation of airborne ScV-L-BC

Aspiration interval hours	Aspirated virus particles units/m^3	Sampling time post aspiration	Recovered virus particles units/m^3 **UVc on**	Aspirated virus particles units/m^3	Sampling time post aspiration	Recovered virus particles units/m^3 **UVc off**
0.0	3.0E+10	0.0	0.0	7.0E+10	0.0	1.0E+03
0.5	4.0E+11	0.5	3.0E+02	1.0E+11	0.5	4.0E+07
1.0	4.0E+11	1.0	4.0E+02	7.0E+12	1.0	3.0E+07
1.5	6.0E+10	1.5	7.0E+02	4.0E+11	1.5	2.0E+08
2.0	5.0E+11	2.0	5.0E+02	7.0E+11	2.0	4.0E+07
2.5	4.0E+12	2.5	7.0E+03	6.0E+11	2.5	9.0E+06
3.0	9.0E+11	3.0	8.0E+03	1.0E+12	3.0	1.0E+07
3.5	8.0E+11	3.5	4.0E+03	8.0E+11	3.5	5.0E+06
4.0	0.0	4.0	7.0E+01	0.0	4.0	7.0E+04
4.5	0.0	4.5	0.0E+00	0.0	4.5	9.0E+02

UVc Off

Graph 5
Reduction of Virus particles over 4.5 hours with no UVc treatment with replacement

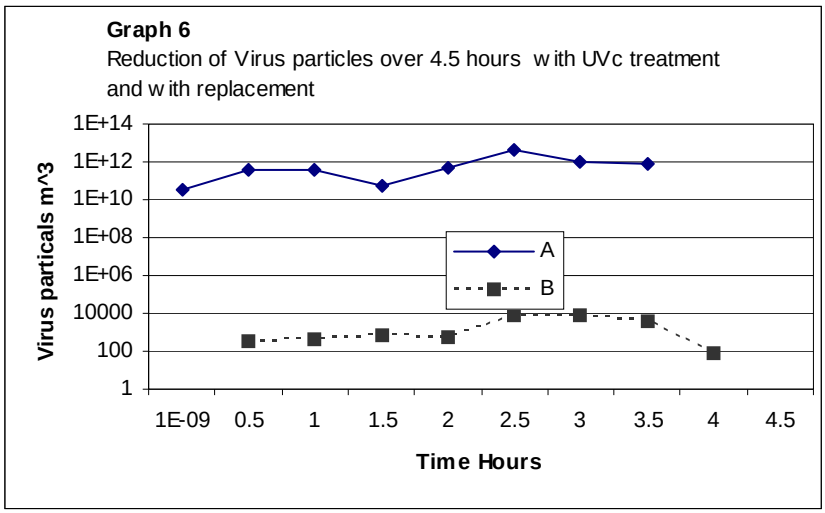


A : Level of Aspirated virus particles per m3 in the pre treatment chamber
B : Level of Aspirated virus particles per m3 in the post treatment chamber UVc off

Summary UVc Off

	Particles	Log
Total particle input over 3.5 hours	1.1E+13	13.0
Total particle recovery over 4.5	3.3E+08	8.5
Depletion UVc off	1.1E+13	4.5

UVC On



A : Level of Aspirated virus particles per m^3 in the pre treatment chamber

B : Level of Aspirated virus particles per m^3 in the post treatment chamber UVc on

Summary UVc On

	Particles	Log
Total particle input over 3.5 hours	7.1E+12	12.9
Total particle recovery over 4.5	2.1E+04	4.3
Correction for Depletion		4.5
Depletion UVc off		4.0

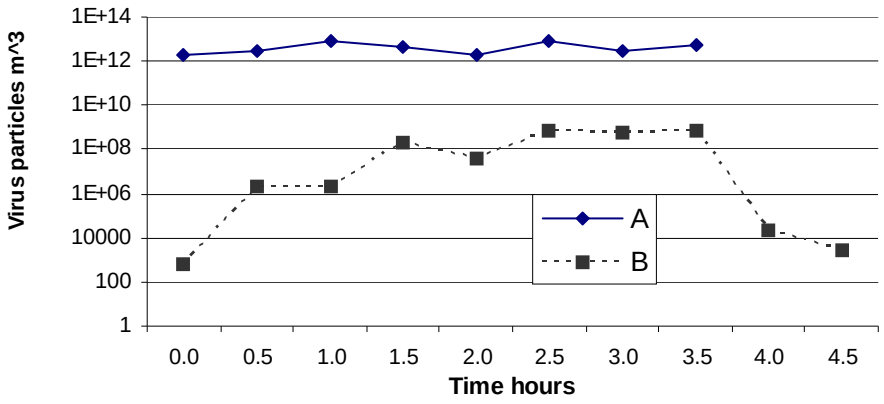
Table 4 Inactivation of airborne fs1 virus

Aspiration interval hours	Aspirated virus particles units/m^3	Sampling time post aspiration	Recovered virus particles units/m^3 **UVc on**	Aspirated virus particles units/m^3	Sampling time post aspiration	Recovered virus particles units/m^3 **UVc off**
0.0	1.0E+12	0.0	0.0	2.0E+12	0.0	7.0E+02
0.5	2.0E+12	0.5	8.0E+02	3.0E+12	0.5	2.0E+06
1.0	7.0E+12	1.0	2.0E+02	8.0E+12	1.0	2.0E+06
1.5	4.0E+11	1.5	8.0E+02	4.0E+12	1.5	2.0E+08
2.0	7.0E+12	2.0	1.8E+03	2.0E+12	2.0	4.0E+07
2.5	3.0E+12	2.5	2.4E+03	9.0E+12	2.5	7.0E+08
3.0	1.0E+12	3.0	1.7E+03	3.0E+12	3.0	6.0E+08
3.5	9.0E+12	3.5	6.0E+02	6.0E+12	3.5	7.0E+08
4.0	0.0	4.0	3.0E+01	0.0	4.0	2.0E+04
4.5	0.0	4.5	0.0E+00	0.0	4.5	3.0E+03

UVc Off

Graph 7

Reduction of Virus particles over 4.5 hours with no UVc treatment and with replacement



A : Level of Aspirated virus particles per m3 in the pre treatment chamber

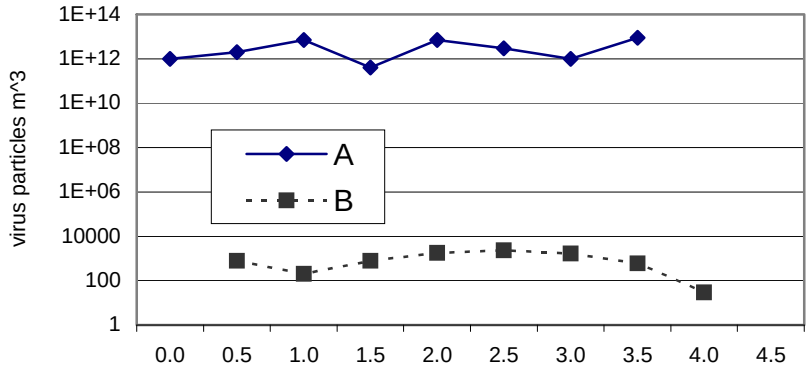
B : Level of Aspirated virus particles per m3 in the post treatment chamber UVc off

Summary UVc Off

	Particles	Log
Total particle input over 3.5 hours	3.7E+13	13.6
Total particle recovery over 4.5	2.2E+09	9.4
Depletion UVc off	3.7E+13	4.2

UVc On

Graph 8
Reduction of Virus particles over 4.5 hours with UVc treatment and with replacement



A : Level of Aspirated virus particles per m^3 in the pre treatment chamber
B : Level of Aspirated virus particles per m^3 in the post treatment chamber UVc on

Summary UVc On

	Particles	Log
Total particle input over 3.5 hours	3.0E+13	13.5
Total particle recovery over 4.5	8.3E+03	3.9
Correction for Depletion		4.2
Depletion UVc on		5.3

Overall Results

Table 5 Summary for all Virus Particles

Corrected mean log reduction of virus over 4.5 hours

Virus	Measured continuous* mean log reduction of virus particles
E.coli T4 Phage	6.1
FCoV[A]	4.4
Saccaharomyces virus ScV-L-BC	4.7
Vibrio phage fs1	5.3

FCoV[A] attenuated non-transmissible variant

* Every 30 minutes over a 4.5 hour period

5.0 Conclusions

During the experiment and subsequent analysis we have been able to demonstrate that inactivation levels across the range of virus particles *not* attributable to UVc irradiation was between 3 and 4.5 log cycles on a mean continuous basis for all virus particles examined.

Such an effect would be anticipated with virus particles *in vitro* and might be due to physiochemical factors operating as a result of aspiration and physical factors within the device considered as a whole. The occurrence and effects of such factors are considered inevitable in laboratory models of environments.

However, our data does demonstrate with a high degree of significance that the Medixair UVc air sterilisation unit was effective in reducing continuous doses of each virus over a 4.5-hour period. The corrected log inactivation data per 30 minute cycle in table 5 clearly illustrates a high and sustained level of inactivation for each viral target, with the range being measured at between 4.4 to 6.1 log reductions of challenge per 30 minutes with dosing at rates described above.

Taking into account the sensitivity of the techniques currently employed for enumerating viral particles it is accurate to state that in this trial, our corrected estimates for kill due to UVc doses in the Medixair device evidences a robust viral inactivation quotient.

" it is apparent that all viable viral targets were inactivated by a greater than 99.999 % efficiency"

Given the level of challenge and taking into account loss of recoverability not due to UVc doses, the significance of the actual inactivation rate attributable to the Medixair unit becomes clearer by considering the percentage kill for each viral target. Deriving this value from table 5 it is apparent that all viable viral targets were inactivated by a greater than 99.999 % efficiency when passed through the Medixair unit. Further this degree of efficiency was demonstrated on a continuous basis during this trial for each viral type.

With the demonstration of such veridical efficiency it is clear that the Medixair unit has immediate application in the sterilisation of atmospheres in environments where airborne viral particles represent a risk of contagion to humans or animals.

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Managing Director
Microsearch Laboratories
June 2003

6.0 References

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