

## Demonstration of AirCleanse™ environmental anti-microbial activity

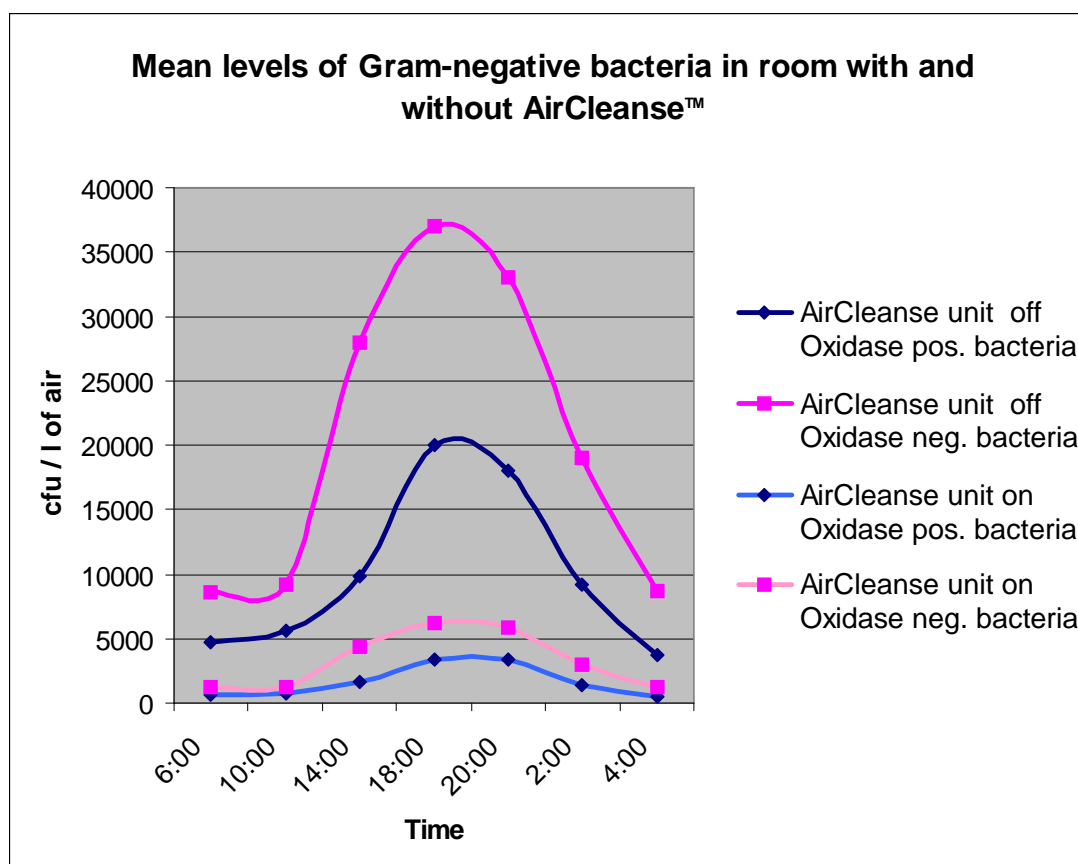
A room used for storing and processing microbiological laboratory waste was used to demonstrate the anti-microbial activity of AirCleanse.

The room, with a volume of 24 m<sup>3</sup>, contained an autoclave and bags of microbiological waste. At any one time the area contained a minimum of 16 untreated waste bags of which between eight to ten bags would be handled and processed in a working day between the hours of 9:00 and 18:00.

Sampling was carried out over a 24-hour period using an air sampling device which samples a known volume of air over a specified time period. The background levels of contamination were determined by sampling over a seven-day period with AirCleanse running, but no filtration and the ozone generator switched off. Subsequently, the test protocol was repeated with the AirCleanse unit fully functioning.

The microbiological samples were cultivated on Violet Red Bile Glucose agar (VRBGA) which is commonly used to recover Gram-negative bacteria for 24 hours at 35°C. It was assumed that all isolates from VRBGA were Gram-negative and all colonies were counted. Colonies were further differentiated on the basis of oxidase reaction. All sampling was done in duplicate.

The graph below shows the results of the tests.



The results show that AirCleanse can produce a high bacterial kill rate in an environment of considerable airborne microbial contamination and where sanitised air is continually becoming recontaminated with Gram-negative bacteria by activities carried out in the room.

Testing carried out by Microsearch Laboratories Ltd.

## Demonstration of AirCleanse™ anti-microbial activity with specific microbial challenge

A series of trials have been carried out where a wide range of different microbial contaminants were continuously introduced directly in to the air intake section of the AirCleanse™ unit for a period of one hour. During the exposure time air samples were collected from the air output section of the unit. The number of viable organisms in the samples were determined.

The table below shows the results of the tests.

Mean concentration of viable micro-organisms introduced to and sampled from operating AirCleanse™ unit

Type of micro-organism	Examples included in the test*	Viable concentration introduced (cfu/m <sup>3</sup> /h)	Viable concentration detected (cfu/m <sup>3</sup> /h)
Gram-negative bacteria	<i>Escherichia coli</i> 0157 H:7	$3.5 \times 10^5$	0
	<i>Salmonella typhimurium</i>	$4.6 \times 10^5$	0
	<i>Pseudomonas aeruginosa</i>	$6.1 \times 10^5$	0
Gram-positive bacteria	<i>Staphylococcus aureus</i> (MRSA)	$4.8 \times 10^5$	0
	<i>S. epidermidis</i>	$3.7 \times 10^5$	0
	<i>Streptococcus pyogenes</i>	$3.6 \times 10^5$	0
	<i>Enterococcus faecalis</i>	$7.3 \times 10^5$	0
Gram-positive spores	<i>Bacillus cereus</i>	$7.1 \times 10^5$	0
	<i>B. megaterium</i>	$6.2 \times 10^5$	90
Yeast	<i>Saccharomyces cerevisiae</i>	$4.3 \times 10^5$	0
Mould (mycelial)	<i>Aspergillus niger</i>	$6.2 \times 10^5$	0
Mould (spore)	<i>Aspergillus niger</i>	$8.2 \times 10^5$	70
Virus (single stranded DNA)	CTX	$4.3 \times 10^{12}$	810
Virus (double stranded RNA)	ScV-L-BC	$9.2 \times 10^{12}$	460
Virus (single stranded positive RNA)	FcoV (attenuated)	$7.1 \times 10^{12}$	300
Virus (double stranded DNA)	T4 Phage	$5.3 \times 10^{12}$	740

\* The number of micro-organisms tested exceeded 35. Only examples of the organisms tested have been included in this table. No viable counts from the air output section were detected with any organism excluded from the results presented.

The results show that AirCleanse™ can inactivate a wide range of micro-organisms including bacterial cells, bacterial spores, mould, mould spores, yeasts and virus particles. Kill rates in excess of Log 12 were obtained consistently for all types of virus particles examined, while for all other types of organisms, no less than a Log 5 kill was obtained.

Testing carried out by Microsearch Laboratories Ltd.

## Safety of AirCleanse™ filter

Electrostatic air filtration is known to produce reduction in the levels of airborne microbial contaminants. A risk associated with stand-alone filtration devices is the accumulation of viable and potentially infective or harmful contaminants within the structure of the filter.

In contrast to such filtration units, AirCleanse™ operates by generating ozone which destroys micro-organisms. The electrostatic filter serves only to capture oxidised debris and removing particulates from the air.

Test were carried out in order to attempt recovery of viable micro-organisms from the interior surface of the AirCleanse™ terminal filter after periods of operation in a room storing and processing microbiological waste. Due to the nature of the activities carried out in this environment, considerable and continuous airborne microbial contamination was present during the operation of the AirCleanse™ unit.

The results are shown in the table below.

Recovery of viable micro-organisms from AirCleanse™ electrostatic filter (cfu/cm<sup>3</sup> of filter)

Length of AirCleanse™ operation	1 day	1 week	1 month
Total viable count	<10	<10	<10
Mould	<10	<10	<10
Yeast	<10	<10	<10
Bacillus spp.	<10	<10	<10
Gram-negative bacterial spp.	<10	<10	<10
Gram-positive bacterial spp.	<10	<10	<10

The data demonstrates that in an environment exhibiting high levels of airborne microbial contamination, there is no detectable recovery of viable micro-organisms in the filter. The used filters are unlikely to represent a biological hazard.

Testing carried out by Microsearch Laboratories Ltd.

## Inactivation of fungi by AirCleanse™

An evaluation of the effectiveness of AirCleanse™ against fungal aerosol challenges was carried out.

Spores from *Neurospora (Chrysonilia) sitophila*, a filamentous fungi such as *Aspergillus*, were spread as an aerosol in a 40m<sup>3</sup> sized room using a nebuliser.  $1.4 \times 10^7$  spores were nebulised over a period of 10 minutes. Room circulation fans (3m/s) were used to disperse the challenge evenly throughout the room. Continuous sampling of the air was carried out using a Mattson-Garvin air sampler and discrete samples were taken every 15 minutes using Oxoid MAQS samplers (200 l). The trial was carried out twice, once with the AirCleanse unit switched off, the second with the unit switched on. The AirCleanse unit was located in the center of the room, one meter above floor level. Malt extract agar (MEA) was used to determine recovery of the mould spores.

*N. sitophila* colonies are difficult to enumerate with agar dishes rapidly becoming overgrown. Results are therefore reported as presence (+) or absence (-) of growth.

The results showed that the airborne spores were removed from the air by the AirCleanse unit within two hours as measured with the Mattson-Garvin air sampler. The results from the MAQS air samplers confirmed this data.

Presence (+) or absence (-) of *N. sitophila* growth with or without AirCleanse in operation

Time (h)	AirCleanse on	AirCleanse off
0 - 1	+	+
1 - 2	+*	+
2 - 3	-	+
3 - 4	-	+

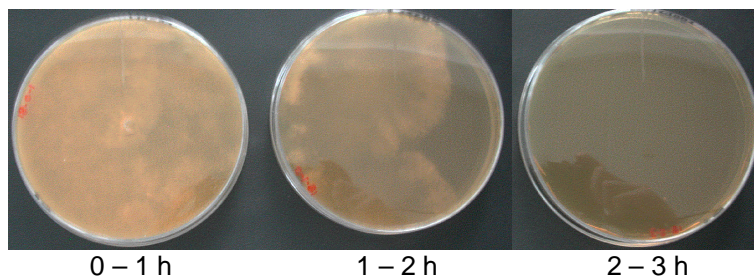
\*reduced growth

With AirCleanse in operation:

Left dish: *N. sitophila* growth 0 - 1 hours after challenge

Centre dish: Reduced *N. sitophila* growth 1 - 2 hours after challenge

Right dish: No *N. sitophila* growth detected 2 - 3 hours after challenge



With the AirCleanse unit switched off, *N. sitophila* growth still occurred in samples taken three to four hours after challenge. After four hours, no further air sampling was carried out.

The results confirm that AirCleanse is effective in removing airborne spores originating from filamentous fungi.

Testing carried out by CCFRA Technology Ltd.