Executive Summary

- Clean Air Ltd manufacture and supply air filtration systems to various corporate and health service organisations world-wide. Glasgow Caledonian University Division of Podiatry was contacted to conduct an independent study into the efficiency and effectiveness of their systems for use in the hospital ward, with specific regard to the influence on Methicillin-Resistant *Staphylococcus aureus* (MRSA). One hospital ward was identified and results were compared before and following activation of the filtration system.

- The sampling was conducted using a portable Surface Air Sampler with samples taken on two days in the ward before and following activation of the filter system. Alongside the air samples, settle plates were also utilised. The hospital ward was a double occupancy ward in the general medical wing of a hospital, situated to the north of Glasgow. The hospital provides a range of acute medical and surgical services for this area of the city.

- Sampling was performed in the morning (9am), lunchtime (12pm) and evening (5pm) with the air samples being collected in duplicate.

- Although *Staphylococcus aureus* was identified within samples from the study, the colonies were confirmed to Methicillin Sensitive (MSSA). MRSA was not identified in any of the samples collected either before or following activation of the filtration system.

- A clear, demonstrable, global reduction in microbial organisms following the activation of the filtration systems is evident. The global results of the study indicate that the filter has a statistically significant effect on microbial counts, with an average decrease of 81% (settle plates) and 24% (air samples). With reference to the settle plates, the effect of the filter varied with time of day; with the volumetric air samples the effect varied both with time of day and day of the week.

Division of Podiatry
School of Health and Social Care
Glasgow Caledonian University
70 Cowcaddens Road
Glasgow
G4 0BA

Report of Study for Clean Air Ltd

The Use of an Air Filtration System in the Hospital Ward - With Specific Reference to its Effect on Methicillin-Resistant *Staphylococcus aureus.*

Authors:

Dr Nichola McLarnon, Research Fellow, School of Health and Social Care [†]

Dr Giles Edwards, Consultant in Pathology[††]

Mr Gordon Burrow, Senior Lecturer, School of Health and Social Care [†]

Dr William MacLaren, Lecturer, School of Computing and Mathematical Science [†]

Dr Kofi Aidoo, Senior Lecturer, School of Life Sciences [†]

Dr Mike Hepher, Reader, School of the Built and Natural Environment [†]

[†] Glasgow Caledonian University, Cowcaddens Road, Glasgow. G4 0BA.

[††] Stobhill Hospital, Balornock Road, Glasgow. G21 3UW

School of Health and Social Care, Caledonian University

Report requested by Clean Air Ltd

An independent Study conducted by Caledonian University in March 2001

Introduction

Clean Air Ltd manufacture and supply air filtration systems to various corporate and health service organisations world-wide. Glasgow Caledonian University Division of Podiatry was contacted to conduct a small-scale study into the efficiency and effectiveness of their systems for use in the hospital ward, with specific regard to Methicillin-resistant *Staphylococcus aureus* (MRSA). With the help of North Glasgow University Hospitals NHS Trust, one hospital ward was identified which would enable a comparison of data before and following activation of the filtration system - a ceiling mounted filtration unit (Model 100C).

MRSA has become a prevalent nosocomial pathogen in hospitals throughout most of the western world. *S. aureus* is the commonest type of bacteria which can infect humans. It is often referred to simply as "Staph" and is a bacterium commonly found on the skin of healthy people. *S. aureus* colonises the skin of humans, leading to localised, superficial, self-limiting abscesses when the skin is disrupted.

It is often found in 20-30% of the noses of normal healthy people and is also commonly found on people's skin. Most strains of this bacterium are sensitive to many antibiotics and infections can be effectively treated. However, *S. aureus,* which are resistant to an antibiotic called Methicillin are referred to as Methicillin-Resistant *S. aureus* or MRSA. Many commonly prescribed antibiotics are not effective against these bacteria. Methicillin-resistant *S. aureus* (MRSA) is resistant to Methicillin and other beta (*ß*) lactamase-resistant penicillins and cephalosporins (Chambers, 1997). Some MRSA strains may also occur in epidemics, indicated by an 'E' before MRSA e.g. EMRSA-16, EMRSA-3 and may be distinguished from others by a number of special laboratory techniques.

The first report of a penicillin-resistant strain of *S. aureus* was published in 1945, revealing its association with penicillinase enzyme produced by the bacteria (Spink & Ferris, 1945). The development of Methicillin antibiotics, synthetic penicillinase resistant penicillin, followed within 2 years. *S. aureus* developed resistance to Methicillin shortly thereafter. MRSA was first reported in the UK and Europe in the 1960's, and in the US in 1968 (Stewart & Holt 1962; Barrett *et al*., 1968). MRSA was recognised as an important nosocomial infection in the U.S. in the late 1960s, and became endemic in some health care settings. *S. aureus* was the most common cause of nosocomial infections reported in 1990-1996 (CDC 1996). The National Nosocomial Infections Surveillance system (NNIS) reports an increasing trend of MRSA, with a 40% increase in resistance in 1999, when compared to 1994-1998 data (NNIS 2000). According to NNIS (2000) MRSA accounts for 52.3% of *S. aureus* nosocomial infections

MRSA is now endemic in many hospitals, and is one of the leading causes of nosocomial pneumonia and surgical site infection and the second leading cause of nosocomial blood stream infections (Boyce, 1994). In hospitals, the most important reservoirs of MRSA are infected or

colonised patients. Although hospital personnel can serve as reservoirs for MRSA and may harbour the organism for many months, they have been more commonly identified as a link for transmission between colonised or infected patients. Colonisation means that MRSA is present on, or in, the body without causing illness. Infection means that MRSA is making the person sick. The main mode of transmission of MRSA is via hands (especially health care workers' hands), which may become contaminated by contact with:

a) Colonised or infected patients,
b) Colonised or infected body sites of the personnel themselves, or
c) Devices, items, or environmental surfaces contaminated with body fluids containing MRSA.

MRSA infection usually develops in hospital patients who are elderly or very sick, or who have an open wound (such as a bedsore) or a tube (such as a urinary catheter) going into their body. The number of people who develop or become infected is not known, but according to some estimates as many as 80,000 patients a year get an MRSA infection after they enter the hospital. The numbers who become colonised is also unknown. Although MRSA is resistant to many antibiotics and often difficult to treat, a few antibiotics can still successfully cure MRSA infections. Patients who are only colonised with MRSA usually do not need treatment, but MRSA can spread to patients who are often very sick with weak immune systems that may not be able to fight off infection. MRSA is almost always spread by physical contact. Hospitals usually take special steps to prevent the spread of MRSA from patient to patient. One of these steps may be to separate, or *isolate*, a patient with MRSA from other patients.

Methodology

Stage 1 Prior to Activation of Filtration Units

Nutrient Agar (NA) and Mannitol Salt Agar (MSA) were prepared in accordance with the manufacturers' instructions - 12.5ml was pipetted into 50mm contact plates and 20ml was poured into 90mm contact plates and the lids immediately replaced. These were then allowed to cool and stored at 4^{o}C until required. The 90mm contact plates prepared in this study were used as settle plates.

Settle plates

Sampling of the indoor mycoflora can be categorised as non-volumetric methods, such as the use of settle or sedimentation plates, and volumetric methods such as the use of commercially available air sampling instruments. Settle plates allow collection of particles large enough only to settle by gravity, however, carefully used settle plates can give an indication of overall airborne organism concentrations (Cruickshank *et al*. 1975). They are an inexpensive alternative to forced flow methods and some regard the method as more representative of the circumstances of infection by dust particles sedimenting into a wound (Clark *et al*. 1985; Buttner & Stetzenbach, 1993). Settle plates offer a semiquantitative analysis of the air, where volumetric methods offer a more quantifiable analysis. Many microbiologists, therefore, regard volumetric air sampling as a superior and more quantifiable method and its use is advocated wherever possible.

Volumetric Air Sampling

Examples of volumetric instruments i.e. methods of drawing measurable amounts of air over an agar plate surface, include the Andersen sampler, the Reuter Centrifugal Air Sampler, the Slit-to-agar Sampler and the Surface Air Sampler. For this study, the poured 50mm contact plates were used in conjunction with a portable Surface Air Sampler (Cherwell Laboratories, Bicester, UK), which draws air at a quoted flow rate of 180 litres per minute onto the surface of the agar plate (Figure 1). This study therefore incorporates and allows a comparison of both of the microbiological techniques - settle plates and volumetric air sampling, adding to the validity of the findings.

Figure 1. Surface Air Sampler (Cherwell Laboratories, Bicester, UK).

Prior to commencement of the study, all linen and soft furnishings within the ward were laundered. The settle plates were left in the same position throughout the study and exposed at the same times as the air sampler. The coverplate of the sampler was removed, one of the agar plates was inserted and the coverplate replaced. The timer was then set to the desired setting - in this case position 2, the equivalent to 40 seconds. The first agar plate was placed into the air sampler and an air sample was taken. Once the time had expired the sampler automatically ceased. The first agar plate was removed and its lid immediately replaced. Subsequent air samples were again taken utilising the same technique.

Samples were taken over two days in the ward, prior to and following activation of the filtration system. Samples were collected at three times of the day: at the start of the day (AM), lunchtime (Noon) and at the end of the afternoon (PM). The volumetric air samples were collected in duplicate. Both the settle plates and the volumetric air samples were marked for time and then incubated (in an accredited laboratory) at 37^0C for 48 hours and then at 25^0C for 5 to 7 days. All of the identification was conducted by an independent third party. An attempt was made to assess the airborne microbial density of the volumetric air samples before and after the activation of the

filtration units. Once the plates were incubated for the required length of time, the number of colonies i.e. a collective mass of millions of micro-organisms (Rose, 1983; Aidoo *et al*., 1995) on each plate was counted and expressed as Colony Forming Units (CFU's).

The Microbial Density (MD) of the air samples was then calculated using the equation:

$$MD = \frac{CFU \times 1000}{60 \times \text{digital setting (2)}}$$

Stage 2 During Use of Filtration Units

Following the second day of testing, the air filtration system was activated. The filtration system is capable of being set to variable speed settings - slow, intermediate and high speed. Throughout the testing, however, the filtration system was set to the slow speed setting. Samples were then taken during the employment of the filtration units using the same methodologies as for the first set of samples.

Results and Discussion

For simplicity the results for the settle plates and air samples will be discussed individually.

Settle Plates

The microbiological composure of the settle plate counts is outlined in Table 1.

			Nutrient Agar				Mannitol Salt Agar		
Filter	**Day**	**Time**	**TC**	**Fg**	**Mc**		**TC**	**Fg**	**Sa**
Before	**Mon**	**AM**	20	1	2		23	0	0
		Noon	11	0	3		6	0	0
		PM	118	1	20		65	0	0
	Wed	**Am**	34	0	3		26	1	0
		Noon	47	3	11		29	3	1
		PM	84	3	10		40	0	0
After	**Mon**	**AM**	31	0	8		30	0	0
		Noon	4	0	6		14	0	0
		PM	0	0	0		0	0	0
	Wed	**Am**	48	0	15		10	0	2
		Noon	22	0	5		11	0	1
		PM	0	0	0		0	0	0

Tc Total colony counts after 36 hours at 37°C, followed by 36 hours at 25°C
Fg Number of colonies of filamentous fungi
Mc Number of colonies of micrococci
Sa Number of colonies of Staphylococcus aureus

Table 1. Microbiological Composure of Settle Plates

With regard to the identification of the fungal species isolated from the settle plate samples, (including both Nutrient and Mannitol Salt Agars) the colonies were mostly *Aspergillus* species. *Aspergillus fumigatus*, a species which is considered to be unacceptable in indoor air and responsible for many of the bronchopulmonary diseases in humans, was not isolated. Although fungal counts are low for the before samples, the filtration system appears to have a marked effect on fungal species, as zero counts are recorded for both agars for all of the post filtration tests. Additionally, a marked difference is recorded for the presence of micrococci in the before and after tests.

Staphylococcus aureus was isolated from the Mannitol Salt Agar, provisionally identified by colonial appearance and colour change. Twenty six colonies were then checked by DNAse, of which 4 were clearly positive and 4 were considered possible (very weak). All four of the clear positives were successfully confirmed (by tube coagulase and repeat DNAse) as Methicillin Sensitive *Staphylococcus aureus*. The four other possible colonies tested negative for tube coagulase and repeat DNAse. No colonies of Methicillin Resistant *Staphylococcus aureus* were isolated.

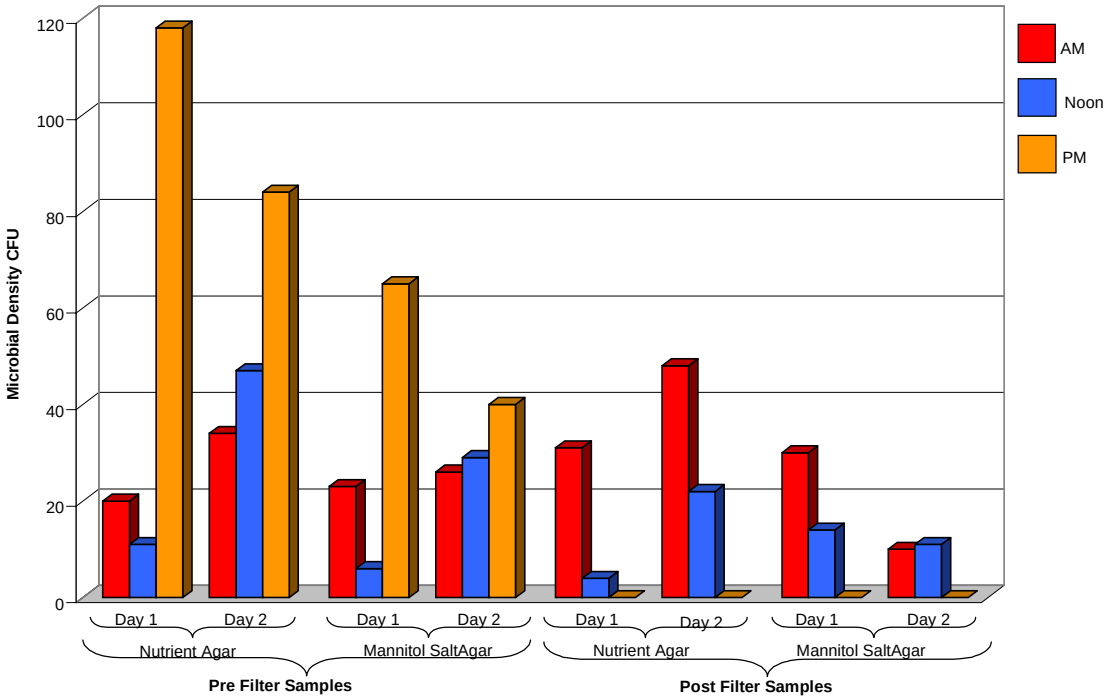

Figure 2. Microbial Density Relating to Filter Activation, Agar Used, Time and Day of Testing (Settle Plates)

Figure 2 clearly demonstrates the global reductions in microbial density for the settle plates following the activation of the filter system. The general trend is a peak microbial density in the afternoon for the pre filter tests, falling to negligible afternoon counts for the post filter tests. The trends for the nutrient agar results are also closely mirrored by those from the Mannitol Salt results.

The data collected from the study was entered into Genstat 5 for statistical analysis (Payne *et al.*, 1993). An analysis of variance was conducted (Scheffé, 1958). The statistical analysis aimed to investigate how the four factors outlined below influenced the true microbial density:

Agar	Nutrient agar (1) and Mannitol Salt agar (2)
Time of Day	Morning (1), mid-day (2) and late afternoon (3)
Filter	Before (1) and after activation (2)
Day of the week	Monday (1) and Wednesday (2)

Table 2 indicates the factors and interaction of factors resulting from the Analysis of Variance, which were shown to have a statistically significant influence upon microbial density, with regard to the settle plates. These factors and their interactions will each be discussed individually. All means discussed are geometric means.

Source of Variation	Degrees of Freedom	Mean Square	
Agar	1	0.52	
Day	1	0.47	
Filter	1	14.69	***
Time	2	2.61	**
Agar.Day	1	0.56	
Agar.Filter	1	0.08	
Agar.Time	2	0.04	
Day.Filter	1	0.16	
Day.Time	2	0.88	*
Filter.Time	2	11.18	***
Residual	9	0.20	
Total	23		

Table 2. Analysis of Variance for Microbial Density of Settle Plates (Natural Log Scale)

*	$p < 0.05$	Evidence of a difference or relationship
**	$p < 0.01$	Strong evidence of a difference or relationship
***	$p < 0.001$	Very strong evidence of a difference or relationship

The ANOVA table with three and four way interactions pooled into the residual (to give an estimate of error as only one sample was obtained), shows that microbial density is significantly influenced by the presence of the Filter and the Time of day, with significant two-way interactions between Filter and Time; and Day and Time. Of particular interest is the effect of the filter, where the ANOVA shows that the main effect (i.e. the effect averaged over the entire experiment) is statistically significant ($p < 0.001$). The estimated geometric means for the samples taken before and after filter activation, and the percent decrease is outlined in Table 3.

Filter	CFU/m^3	% Decrease
Before activation	31.8	81.5
Following activation	5.9	

Table 3. Global Effect of the Filtration System on Microbial Density (Settle Plates)

However, this summary is incomplete due to the significant two-way interaction between Filter and Time of Day. Further exploration of the significant interaction between Filter and Time of Day (Table 4) suggests that the filter has the greatest effect on microbial counts in the evening. The

effect of the filter is not statistically significant in the morning or at noon, but is statistically significant for the afternoon counts ($p < 0.001$). It is possible, however, that the filter has a greater influence on the microbial density when baseline air counts are high and not such a marked effect when counts are low. This observation supports earlier findings by the authors regarding the use of the same filtration system in podiatry clinics. None of the three way interactions were significant.

Time	**AM**		**Noon**		**PM**	
Filter	**Before**	**After**	**Before**	**After**	**Before**	**After**
	25.2	26	17.6	11.0	71.3	0.0
% Decrease	3.2 increase		37.5		100.0	

Table 4. Effect of Filter and Time on CFU (Settle Plates)

Volumetric Air Samples

The microbiological composure of the air sample counts is outlined in Table 5.

Agar			**Nutrient Agar**							**Mannitol Salt Agar**					
			TC		**Fg**		**Mc**			**TC**		**Fg**		**Sa**	
Repeat Measurement			**1**	**2**	**1**	**2**	**1**	**2**		**1**	**2**	**1**	**2**	**1**	**2**
Filter	**Day**	**Time**													
Before	**Mon**	**AM**	21	18	0	0	6	10		13	15	0	0	0	0
		Noon	13	13	0	0	3	5		6	9	0	0	0	0
		PM	16	18	0	0	9	5		15	15	0	0	0	0
	Wed	**Am**	10	13	0	1	4	3		10	15	1	0	0	0
		Noon	25	31	17	10	3	5		28	23	10	13	0	0
		PM	15	13	2	4	6	3		9	13	0	0	0	0
After	**Mon**	**AM**	6	7	0	0	3	3		8	2	0	0	0	0
		Noon	14	16	0	1	6	4		6	10	0	0	0	0
		PM	13	16	0	1	4	9		17	10	0	0	0	0
	Wed	**Am**	54	42	0	0	30	17		25	36	0	0	0	0
		Noon	8	13	0	0	2	2		19	14	0	0	0	0
		PM	5	5	0	0	2	1		9	3	0	0	0	0

Table 5. Microbiological Composure of Volumetric Air Samples

Tc Total colony counts after 36 hours at 37°C, followed by 36 hours at 25°C
Fg Number of colonies of filamentous fungi
Mc Number of colonies of micrococci (colonial morphology only)
Sa Number of colonies of *Staphylococcus aureus*

With regard to the identification of the fungal species isolated from the air samples, (including both Nutrient and Mannitol Salt Agars) the colonies were again mostly *Aspergillus* species. No *Staphylococcus aureus* colonies were isolated from the Mannitol Salt agar, either before or after activation of the filtration system. As with the settle plate samples, fungal counts are low for the before samples. Again however, the filtration system appears to have a marked effect on fungal species, as zero counts are recorded for both agars for all of the post filtration tests.

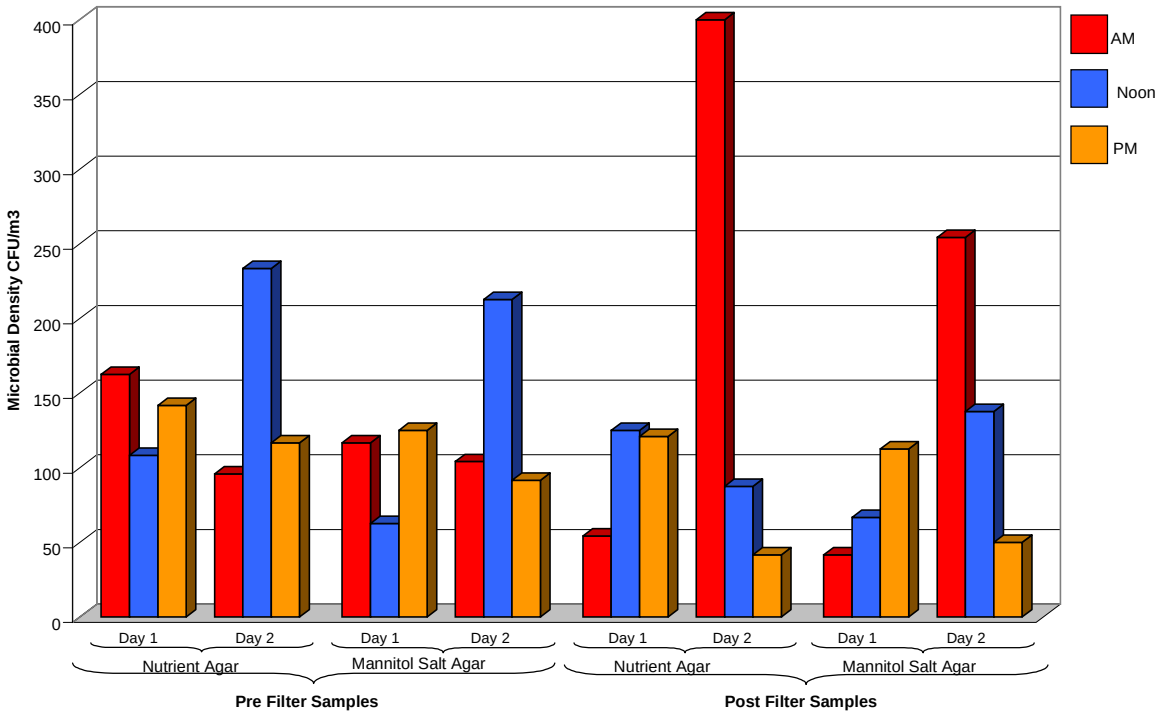

Figure 3. Microbial Density Relating to Filter Activation, Agar Used, Time and Day of Testing (Volumetric Air Samples)

Figure 3 demonstrates the global reductions in microbial density for the air samples following the activation of the filter system. Although there is apparently no distinctive trends for this section of the study, the nutrient agar results (similar to the settle plates results) are closely mirrored by those from the Mannitol Salt results. One striking observation from Figure 3 is the sharp peak measured on Wednesday morning (Day 2), following activation of the filtration system. A possible explanation for this observation, which has previously been reported to influence air counts, was the changing of bed linen in the ward during the collection process (Shiomori *et al*., 2002).

Again as for the settle plates, the results were entered into Genstat 5 for statistical analysis and an analysis of variance conducted. The statistical analysis again aimed to investigate how the five factors below influenced the microbial density.

Agar	Nutrient agar (1) and Mannitol Salt agar (2)
Time of Day	Morning (1), mid-day (2) and late afternoon (3)
Filter	Before (1) and after activation (2)
Day of the week	Monday (1) and Wednesday (2)
Repeat Measurement	1st measurement (1) and 2nd measurement (2)

Table 6 again indicates the factors and interaction of factors resulting from the Analysis of Variance, which were shown to have a statistically significant influence upon microbial density, with regard to the volumetric air samples. These factors and their interactions will again be discussed individually. All means discussed are geometric means.

Source of Variation	Degrees of Freedom	Mean Square	
Agar	1	0.52	*
Day	1	0.86	**
Filter	1	0.93	**
Time	2	0.32	
Agar.Day	1	0.35	
Agar.Filter	1	0.00	
Day.Filter	1	0.21	
Agar.Time	2	0.04	
Day.Time	2	2.45	***
Filter.Time	2	0.26	
Agar.Day.Filter	1	0.02	
Agar.Day.Time	2	0.17	
Agar.Filter.Time	2	0.10	
Day.Filter.Time	2	3.23	***
Agar.Day.Filter.Time	2	0.07	
Residual	24	0.10	
Total	47		

Table 6. Analysis of Variance for Microbial Density of Volumetric Air Samples (Natural Log Scale)

*	$p < 0.05$	Evidence of a difference or relationship
**	$p < 0.01$	Strong evidence of a difference or relationship
***	$p < 0.001$	Very strong evidence of a difference or relationship

Of particular interest again is the effect of the filter, where the ANOVA shows that the main effect (i.e. the effect averaged over the entire experiment) is statistically significant ($p = 0.006$). The estimated geometric means for the samples taken before and after filter activation, and the percent decrease is outlined in Table 7.

Filter	CFU/m^3	% Decrease
Before activation	122.1	24.2
Following activation	92.5	

Table 7. Global Effect of the Filtration System on Microbial Density (Volumetric Air Samples)

This summary of the effect of the filter is, however, incomplete, in that the effect of the filter is influenced by Day and Time of Day. Unlike the results for the settle plates, one of the three-way interactions - Day, Filter and Time was statistically significant ($p < 0.001$). This effect is illustrated in Table 8, showing a three-way table of estimated geometric means indexed by Day, Filter and Time.

Filter	Before			After		
Time of Day	**AM**	**Noon**	**PM**	**AM**	**Noon**	**PM**
Day 1	137.3	81.4	133.0	42.4	89.7	114.3
Day 2	98.5	221.4	102.4	315.1	107.4	42.5
% Decrease	28.3	172 increase	23.0	643.2 increase	19.7 increase	62.8

Table 8. Effect of Day, Filter and Time on CFU/m^3 (Volumetric Air Samples)

The measurements taken on Monday (Day 1) mornings were lower on average following activation of the filtration system (28% decrease); while those taken on Wednesday were considerably higher (643% increase). However, as discussed previously, this may be explained due to the changing of the bed linen in the ward prior to the results being taken. The filter also seemed to have little effect on microbial counts on noon readings on Monday, although appeared to reduce the counts on Wednesday. Both Monday and Wednesday evening measurements were reduced (23% and 63% respectively).

The ANOVA also illustrates a statistically significant difference between the Nutrient Agar and the Mannitol Salt Agar ($p = 0.035$). The estimated geometric means for the Nutrient agar and Mannitol Salt agars were 117.9 and 95.8 CFU/m^3 respectively. However, none of the interaction terms involving the agars were statistically significant at the 5% level. Additionally, there were no systematic differences between the 1st and 2nd (repeat) samples. This was confirmed through the use of a paired t-test ($p = 0.844$), which illustrates the validity and reliability of the volumetric air sampler used in the study.

One point to be borne in mind regarding the study as a whole, is that although trying to keep the environment of the ward as controlled as possible (with the door of the ward shut at all times), the authors had no control over proceedings when not on site. Temperature and relative humidity readings were recorded for all the days under test (Figure 4). The range of temperatures recorded over the test period was narrow and lay between 23.6°C and 26.9°C, with a mean reading of 24.7°C. The range of relative humidity readings recorded over the test period was again quite narrow, and lay between 30 and 42%, with a mean reading of 37.4%.

The lowest temperature readings i.e. Day 1 of the post filter results, can be accounted for in that one of the patients had, due to excessive heat within the ward, requested that the door remain ajar. In addition, a free-standing electric fan was also utilised. The higher temperature readings recorded during the testing period can perhaps be explained by the presence of visitors within the ward. On a number of occasions, particularly during the final day of testing at least 4 people were present i.e. 2 patients and 2 visitors.

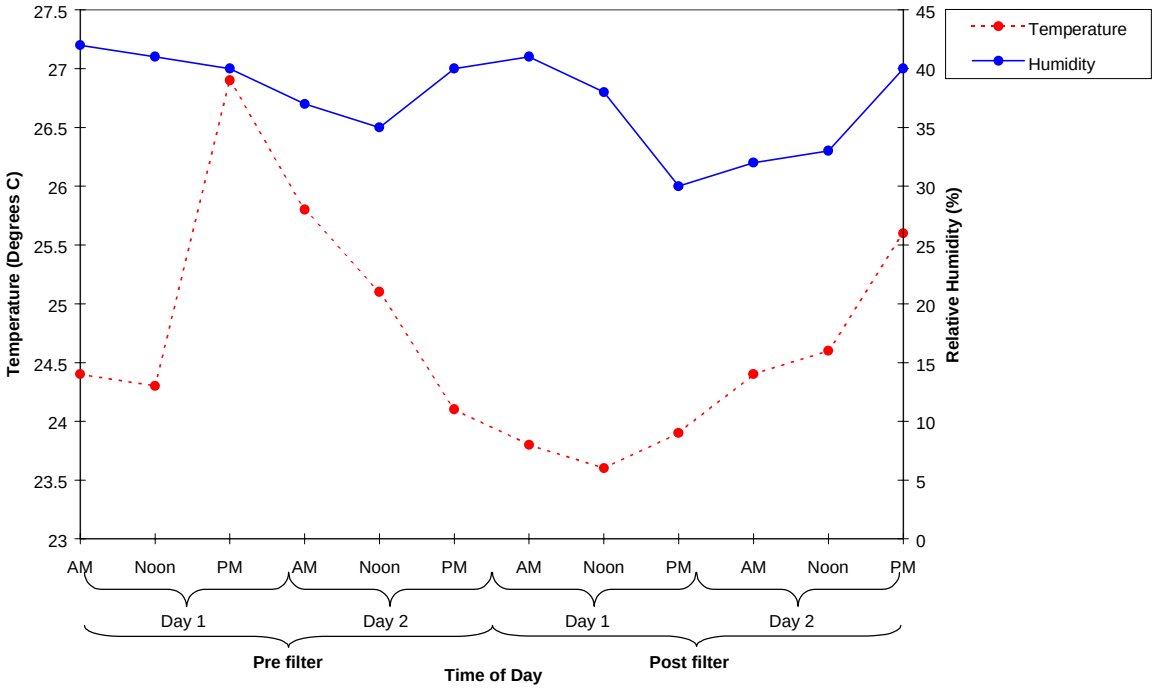

Figure 4. Temperature and Humidity Readings from Ward Prio to and During Activation of Filtration System

Contamination of Curtains Removed from Hospital Ward Prior to Commencement of Study

Previous research into the area of ward curtain contamination has shown that they can support various types of bacteria, including MRSA (Das *et al*., 2002; Palmer 1999). This contamination may be a significant factor in the spread of nosocomial infection. Therefore, in order to complete the study, an attempt was made to measure the contamination of the curtains, which were removed for laundering prior to commencement of the study. A 50mm contact plate, of both Nutrient and Mannitol Salt Agar, was placed randomly against the material of each of the ten curtains. The plates were marked and then incubated (in an accredited laboratory) at 37^0C for 48 hours and then at 25^0C for 5 to 7 days. All of the identification was conducted by an independent third party. The results are outlined in Table 9.

		Nutrient Agar			Mannitol Salt Agar		
Plate number	**Area**	**Tc**	**Fg**	**Mc**	**Tc**	**Fg**	**Sa**
1	Window	5	0	0	0	0	0
2	Corridor	5	0	0	1	0	0
3	Cubicle	3	0	1	3	0	0
4	Cubicle	13	0	6	4	0	0
5	Cubicle	0	0	0	1	0	0
6	Cubicle	4	0	0	0	0	0
7	Window	5	0	1	4	0	0
8	Corridor	1	0	0	0	0	0
9	Corridor	3	0	2	1	0	1
10	Corridor	2	0	1	0	0	0

Tc Total colony counts after 36 hours at 37°C, followed by 36 hours at 25°C
Fg Number of colonies of filamentous fungi
Mc Number of colonies of micrococci (colonial morphology only)
Sa Number of colonies of *Staphylococcus aureus*

Table 9. Contamination of Ward Curtains

Staphylococcus aureus was isolated from the Mannitol Salt Agar, provisionally identified by colonial appearance and colour change. Four colonies were then checked by DNAse, of which 1 was clearly positive. The clear positive was successfully confirmed (by tube coagulase and repeat DNAse) as Methicillin Sensitive *Staphylococcus aureus*. The 3 other possible colonies tested negative for tube coagulase and repeat DNAse. No colonies of Methicillin Resistant *Staphylococcus aureus* were isolated. The most contaminated area was the curtain surrounding the cubicle. This was to be expected, in that this is the part that is most frequently handled when the curtains are being opened and closed. The other results yielded relatively low counts.

Conclusions

MRSA was not detected within the present study. However, despite the variations in reductions of microbial density, as highlighted in the Analysis of Variance for both the settle plates and the air samples, the global results of the study indicate that the filter has a statistically significant effect on microbial counts. The average decrease was 81% (settle plates) and 24% (air samples) and overall the filter reduced the geometric mean airborne density by a factor of 0.18 (settle plates) and 0.76 (air samples).

Disclaimer

The information contained in this report was conducted without any influence, financial or otherwise, from Clean Air Ltd and the authors of this report did not receive any form of remuneration for the study, ensuring independence of the study design, results and conclusions.

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Statistical Methodology

Air sampling was carried out on **two days** (Monday and Wednesday), using **two agars** before **filter** units were activated, and on two days (Monday, Wednesday of the following week) after the **filter** was activated. On each sampling day at each clinic, sampling was performed at three **times** of day (morning, mid-day and late afternoon). With the volumetric air samples **two measurements** were obtained for each condition.

Table 10 shows how the cross-classification of the **four** factors: Time, Agar, Filter, Day (of the week) gives rise to the 72 observations collected.

Time	Agar	Filter			
		Before		After	
		Day		Day	
		Monday	Wednesday	Monday	Wednesday
AM	Nutrient				
	Mannitol Salt				
Mid-day	Nutrient				
	Mannitol Salt				
PM	Nutrient				
	Mannitol Salt				
AM	Nutrient				
	Mannitol Salt				
Mid-day	Nutrient				
	Mannitol Salt				
PM	Nutrient				
	Mannitol Salt				
AM	Nutrient				
	Mannitol Salt				
Mid-day	Nutrient				
	Mannitol Salt				
PM	Nutrient				
	Mannitol Salt				

Table 10. Cross-classification of the four factors: Time, Agar, Filter, Day (of the week)

Response variable

The response variable for the settle plates is the natural log (CFU + 1) of microbial density (per litre of air), because of the zero counts. However, for the air samples, the response variable is the natural log (CFU). This was derived from counts of colonies.

The reason for using a transform is described below.

Statistical Analysis

The statistical analysis aimed to investigate how the four factors (Time, Agar, Filter, and Day of the week) influenced the true microbial density. The method used was Analysis of Variance (Scheffe, 1958). According to this method, the total variation of the response variable is measured by the sum of squares of the deviations from the mean (transformed) microbial density. This total sum of squares is partitioned into component sums of squares each of which measures the effect of each factor, or 'interaction between factors' upon the response variable. (Sometimes factors act in combination with one another to influence the response variable. When this happens, it can be said that the factors are *interacting* with each other. Interactions can be between two factors, three factors, and so on). The results of an Analysis of Variance are displayed in a so-called Analysis of Variance Table (see Table 2 of the main report).

Associated with each sum of squares is a whole number known as the degrees of freedom (DF) of the sum of squares. For sums of squares corresponding to actual factors (*i.e*. not to interactions) the DF is one less than the number of levels of the factor. Hence, for example, the DF for Time of Day is 1. For interactions of 2 or more factors, the DF for each factor involved in the interaction is multiplied to obtain the DF for the interaction.

The third column of the Analysis of Variance Table, is a column of mean squares, whose entries are given by the sums of squares column divided by the DF column.

In the present analysis, there are 4 factors whose influence is to be examined. Thus, potentially, an analysis of variance table could contain interactions including up to 4 factors. However, if all interactions are included in the analysis of variance table, tests of statistical significance cannot be carried out. What has been done here is to assume that interactions of 4 factors will be negligible, and to regard the combined sum of squares for these interactions as a measure of random variation.

The statistical significance of each term in the analysis of variance table is tested by dividing its mean square by the residual mean square, the latter having been obtained from a combination of all 3-factor interactions, and the single 4-factor interaction. This gives a set of so-called variance ratios, which are referred to tables of the F-distribution to test each factor and each interaction for statistical significance.

Use of the log-scale for analysis

The random variability of a response variable based on counts usually increases with the mean value of the count. This contradicts one of the requirements of the analysis of variance, namely that the magnitude of random variation is constant. This lack of constant variance can be dealt with by applying a transform to the response variable. In this case, the natural log transform was used, a frequent choice in this situation.

With the predicted geometric means for the settle plates, since logs of zero cannot be taken, 1.0 has been added to every microbial density before taking logs. This means that predicted means can be obtained from the analysis of variance model: *exp* (predicted mean) – 1.0. These predicted means are approximately equal to geometric mean microbial densities. For the air samples the predicted mean was used (as no zero counts were recorded).