

Executive Summary

- Clean Air Ltd manufacture and supply air filtration systems to various corporate and health service organisations worldwide. Glasgow Caledonian University Division of Podiatry was contacted to conduct a small-scale independent study into the efficiency and effectiveness of their systems for use in podiatry clinics. Three clinics were identified enabling comparison of data between podiatry clinics.
- Podiatrists often experience non-specific symptoms such as headaches, eye and throat irritations, chest tightness and shortness of breath, and fatigue. Air-borne contaminants include commonly used chemicals, microbial organisms, fibrous glass particles, and dust some of which have been clearly identified in podiatric clinics as occupational health hazards (McLarnon, 2000).
- The sampling was conducted using a portable Surface Air Sampler was conducted with samples taken on two days at three sites before and after installation of the filtration units. The three sites were single occupancy chiropody rooms, the largest room being a biomechanics/ orthotics manufacturing laboratory. All three rooms were in modern (1960's-70's) Health Centres, specifically used for podiatry treatments on a daily basis. Sampling was performed at the start of the day before any patient treatments, at lunchtime, at the end of the morning session and at the end of the afternoon session.
- Samples were collected in duplicate, one of the general circulating air, away from the workstation/podiatrist and one in the breathing zone of the podiatrist. A validated questionnaire was also used to collect subjective evidence, which was designed to account for specific problems related to Sick Building.
- A clear, demonstrable, global reduction in microbial organisms following the installation of the filtration systems is evident. The influence of the filtration system and the time of day is evident for all three clinics tested. The position of the air sampler i.e. whether in the region of the podiatrist's face or in the background does not affect the number of microbial organisms found. The filter has the greater influence on the microbial count when baseline air counts are high i.e. the higher the incidence of microbes in the air the greater the effect the filter system has. The global results of the study indicate the filter has a statistically significant effect on microbial counts, with an average percentage decrease of 65%. Seventy-five percent of practitioners noticed a difference in the environment of the treatment rooms, these being described as perceptible differences to the air – being cooler, fresher, less 'stuffy' and less 'stagnant'. When asked if they would wish such a unit to be available to them, 63% of the practitioners stated they would.
- Although noise was seen as a factor this should be viewed in the light of the type of clinics chosen for the sample. The clinics are generally very quiet with little background noise. Thus, any machine making a noise will be viewed as noisy but it did not interfere with normal conversation between practitioners and patients; and 6/8 practitioners after a few days forgot they were there.
- The filter units reduce airborne contaminants making the clinics cooler and less stuffy, while enhancing the environment by reducing the level of airborne organisms likely to lead to infections for both practitioners and patients.

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Report of Study for Clean Air Ltd

The Use of an Air Filtration System in Podiatry Clinics

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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 worldwide. Glasgow Caledonian University Division of Podiatry was contacted to conduct a small-scale study into the efficiency and effectiveness of their systems as used in podiatry clinics. With the help of Greater Glasgow Primary Care NHS Trust, three clinics were identified which would enable a comparison of data between the podiatry clinics.

“Indoor air quality has deteriorated so much since the 1970s oil shortage and subsequent energy-efficient construction of buildings that people are becoming seriously ill by just breathing the indoor air. This is a problem with all industrial buildings and hospital staff are at particular risk” (Brownson, 2000).

The sick building syndrome (SBS) is a term mainly applied to irritative symptoms in the eyes, skin and upper airways that are experienced in certain environments.

Sick building syndrome has various aetiological factors ranging from temperature, humidity, and air movement to internal pollutants, dust, lighting, and noise factors according to Niven *et al.*, (2000). According to one study almost 25 million workers in 1.2 million commercial buildings in the United States have symptoms of SBS and it is a source of ocular discomfort exacerbating the successful wear of contact lenses (Backman & Haghghat, 1999). Ocular discomfort could be an indicator of poor indoor-air quality and sick building syndrome and this is one area reported and investigated recently by podiatrists as being a problem (McLarnon, 2000).

Other studies suggest, *“polluted indoor air has been associated with health problems that include asthma, sick building syndrome, multiple chemical sensitivity, and hypersensitivity pneumonitis”* (Oliver & Shackleton, 1998). Symptoms are often non-specific and include headache, eye and throat irritation, chest tightness and shortness of breath, and fatigue. Air-borne contaminants include commonly used chemicals, microbial organisms, fibrous glass particles, and dust. Again dust has been clearly identified in recent research in podiatric clinics as an occupational health hazard (McLarnon, 2000).

Identified causes include defective building design and construction, aging of buildings and their ventilation systems, poor climate control, inattention to building maintenance. A major contributory factor, according to Oliver & Shackleton (1998) is the explosion in the use of chemicals in building construction and furnishing materials over the past four decades.

In industrialized countries such as the UK, about 90% of the people’s time is spent indoors. The ambient factors affecting indoor thermal comfort are:

- Air temperature and humidity,
- Air velocity, and
- Radiant heat exchange within an enclosure.

In assessing the thermal environment, consideration of all ambient parameters, the insulating properties of the occupants' clothing, and the activity level of the occupants by means of heat balance models of the human body requires consideration (Hoppe, 1993).

Apart from thermal parameters, air quality (measured and perceived) is also of importance for well-being and health in indoor environments. Pollutant levels are influenced by both outdoor concentrations and by indoor emissions. Indoor levels can thus be lower (e.g. in the case of ozone and SO₂) or higher (e.g. for CO₂ and formaldehyde) than outdoor levels.

The sensation of dryness and irritation is essential in SBS and such symptoms are common in both office and hospital employees.

The humidity of the ambient air has a wide range of effects on the energy and water balance of the body, including elasticity, air quality perception, build-up of electrostatic charge and the formation of mould. However, its effect on the indoor climate is often overestimated. While air-handling systems are commonly used for achieving comfortable indoor climates, their use has also been linked to a variety of problems, some of which have received attention within the context of "sick building syndrome". Although the cause is unknown, there is evidence that the local environment of the workstation is an important determinant of symptoms (Menzies *et al.*, 1997).

Some studies investigated the symptoms of hospital personnel in association with perceived indoor air quality. The symptoms experienced by the personnel were measured before and after the study period by a standardised self-administered questionnaire (Nordstrom, 1994). Other studies report that although it is often suggested that symptoms in office workers are due to circulating microorganisms or particles, epidemiological studies investigating the relationship between them have been lacking. Harrison *et al.* (1992) undertook a cross-sectional study which combined medical and aerobiological assessments of offices in Great Britain and found that, although airborne particulates and micro-organisms were unlikely to be the sole cause of sick building syndrome, there were positive associations between symptom prevalence rates and levels of airborne viable bacteria and fungi within groups of buildings with similar ventilation systems. They therefore concluded that this association suggested a possible causal link that should be explored.

Clearly, in any building, a major priority must be to provide and maintain an environment conducive to occupant health and well-being. In air-conditioned buildings indoor air quality is closely dependent on the efficiency of air-conditioning and humidifier systems since these systems provide a suitable environment for the proliferation of microorganisms. Pollutants released by microorganisms are termed 'bioaerosols' and may be spread in the indoor environment through the air-conditioning system (Rossi *et al.*, 1991).

With this background in mind the authors undertook a small-scale study of three podiatry clinics in the Greater Glasgow Area. For reasons of confidentiality the clinical sites and personnel involved have been anonymised although the authors would like to thank the clinicians involved but also the support of the Head of Profession (Mr. Jamie Quin) and the Sector Manager of the podiatry services (Mr Alistair Hunter).

Methodology

Stage 1 Prior to Installation of Filtration Units

Nutrient agar was prepared in accordance with the manufacturers' instructions - 12.5ml was pipetted into 50mm contact plates and the lids immediately replaced. These were then allowed to cool and stored at 4°C until required. Air sampling was conducted using a portable Surface Air Sampler (Cherwell Laboratories, Bicester, UK), which draws air at a quoted flow rate of 180 litres per minute onto the agar surface (Figure 1).

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.



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

Samples were taken on two days at the three sites prior to installation of the filtration units. The three sites were single occupancy chiropody rooms with the details described in Table 1. The largest room was a biomechanics/orthotics-manufacturing laboratory. All three rooms were in modern (1960's-70's) Health Centres but were specifically used for podiatry treatments on a daily basis. Various clinicians used the rooms and they were therefore not utilised by only one individual podiatrist over the week period.

Clinic	Room Dimensions			
	Length (m)	Breadth (m)	Height (m)	Volume (m ³)
1	3.66	4.42	2.44	39.47
2	6.24	4.47	2.38	66.38
3	4.57	3.58	2.82	46.13

Table 1. Dimensions of the three treatment rooms.

Air samples were collected at three times of the day:

- At the start of the day before any patient treatments
- At lunch time, at the end of the morning session
- At the end of the afternoon session

Samples were collected in duplicate:

- One of the general circulating air, away from the work-station
- One in the breathing zone of the podiatrist

These samples were marked for time and location and then incubated, alongside two agar plates which were not inoculated, thus serving as controls. The plates were incubated at 37⁰C for 48 hours and then at 25⁰C for 5 to 7 days.

An attempt was made to assess the airborne microbial density before and after the installation 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 & Barron, 1983; Aidoo *et al.*, 1995) on each plate was counted and expressed as Colony Forming Units (cfu's).

Microbial Density (MD) was then calculated as:

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

Stage 2 During Use of Filtration Units

Samples were taken during the employment of the filtration units using the same methodologies as for the first set of samples.

Stage 3 Questionnaire to Staff

A questionnaire (Appendix 1), designed to account for specific problems related to Sick Building Syndrome was used in this study. The questionnaire has been validated and has been used in previous studies. It originated from an Indoor Air Quality Complaint Form (Environmental Protection Agency, 1991).

The questionnaire was left for each occupant (podiatrist) to complete after experiencing a few days of the filtration units *in situ* in the workplace.

Results and Discussion

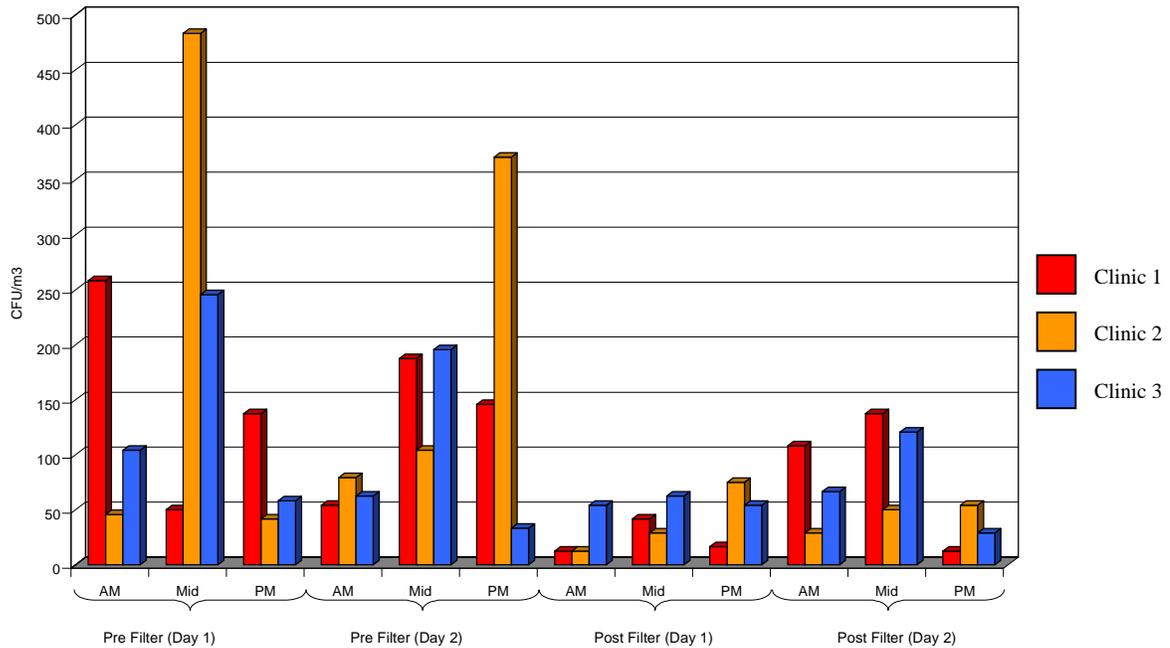


Figure 2. CFU/m³ in Relation to Filter Placement, Clinic and Time of Testing.

Figure 2 clearly demonstrates the global reductions in microbial density for the tests following the placement of the filtration systems. The influence of the filtration system and the time of day, can be clearly seen for each of the three clinics tested. The general trend for Clinics 1 and 3 is a peak microbial density following the end of the morning session. This is apparent for both pre and post filter tests. The general trend for clinic 2 with regards to microbial density is a steady increase towards a peak density in the afternoon. The higher microbial density for the pre filter tests can possibly be explained in that this clinic was primarily used for the manufacture of orthotics/ insoles. These types of clinics generally tend to create more dust and airborne particles due to grinding processes. Other explanations for these patterns are discussed in the following section.

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

Clinic	1, 2 and 3
Time of Day	Morning (1), mid-day (2) and late afternoon (3)
Position	Level of Podiatrist's face (1) and background (2)
Filter	Before and After installation
Day of the week	Monday (1) and Thursday (2)

Details of the statistical methods employed are outlined in Appendix 2.

Results

Source of Variation	Degrees of Freedom	Mean Square	
Clinic	2	0.23	
Day	1	1.73	
Time	2	3.97	**
Position	1	0.13	
Filter	1	19.68	***
Clinic x Day	2	0.59	
Clinic x Time	4	2.08	*
Day x Time	2	0.51	
Clinic x Position	2	1.48	
Day x Position	1	0.02	
Time x Position	2	1.35	
Clinic x Filter	2	2.40	*
Day x Filter	1	1.14	
Time x Filter	2	0.08	
Position x Filter	1	0.57	
Clinic x Day x Time	4	1.73	
Clinic x Day x Position	2	0.09	
Clinic x Time x Position	4	0.35	
Day x Time x Position	2	1.37	
Clinic x Day x Filter	2	0.22	
Clinic x Time x Filter	4	1.91	*
Day x Time x Filter	2	3.85	*
Clinic x Position x Filter	2	0.42	
Day x Position x Filter	1	1.17	
Time x Position x Filter	2	0.55	
Residual	20	0.63	
Total	71		

Table 2. Table of Analysis of Variance of Microbial Density (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

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.

From the results of the aerobiology study, the microbial density is therefore significantly influenced by the presence of the filter, the time of day, the clinic being tested and the day on which the testing takes place. The position of the air sampler i.e. whether in the region of the podiatrist's face or in the background does not appear to have an effect on microbial density. These factors and their interactions will each be discussed individually. All means discussed are geometric means.

% Decrease
65.7

Table 3. Percentage Decrease in Microbial Density upon Placement of Filtration System.

	Before (CFU/m³)	After (CFU/m³)
Filter	102	35

Table 4. Global Effect of the Filtration System on Microbial Density.

Table 3 illustrates the percentage decrease in microbial density following installation of the units. This decrease has also been shown to be statistically significant. Table 4 illustrates the global effects of the filter system on microbial density. The geometric mean microbial density can be seen to be 102 CFU/m³ prior to the installation of the filtration units, falling to a mean of 35 CFU/m³ following installation of the units.

Clinic	% Decrease
1	77.8
2	75.0
3	27.8

Table 5. Percentage Decrease in Microbial Density by Clinic Tested.

Clinic	Filter	
	Before (CFU/m³)	After (CFU/m³)
1	117	26
2	116	29
3	79	57

Table 6. Joint Effect on Microbial Density of Filter Placement and Clinic Tested.

Table 5 illustrates the percentage decrease in microbial density and Table 6 illustrates how this global result varies by clinic.

It can be seen that the results for microbial density for Clinics 1 and 2 are similar to those of Table 3 i.e. the global effects. Clinic 3 does not demonstrate as marked an influence. However, this may be due to differences in the numbers of patients treated, types of podiatric treatments being conducted and the presence of an additional internal air filtration system.

Clinic	Time		
	1	2	3
	% Decrease	% Decrease	% Decrease
1	69.5	38.5	94.3
2	81.7	83.6	50.4
3	5.0	58.2	5.1

Table 7. Percentage Decrease in Microbial Density by Clinic and Time of Testing.

Clinic	Time Filter	1		2		3	
		Before (CFU/m ³)	After (CFU/m ³)	Before (CFU/m ³)	After (CFU/m ³)	Before (CFU/m ³)	After (CFU/m ³)
1		118	36	96	59	140	8
2		60	11	220	36	119	59
3		60	57	208	87	39	37

Table 8. Joint Effect on Microbial Density of Combination of Filter Placement, Clinic and Time of Testing.

Table 7 indicates the percentage decrease in microbial density as a result of this three-way combination of factors. Table 8 indicates the results of a three-way combination of factors - where microbial density is influenced by the placement of the filtration system *and* the clinic being tested *and* the time of testing. The results indicate a significant overall reduction in microbial density, with clinics 2 and 3 demonstrating a daily peak in density at the end of the morning session.

It can also be seen that the change in microbial density with regards to Clinic 3, particularly following the morning and afternoon sessions, is not as marked as in the other 2 clinics upon installation of the filter. However, this may be due again to differences in the numbers of patients treated, types of podiatric treatments being conducted and the presence of an additional internal air filtration system. The filter appears to have a greater influence on the microbial density when baseline air counts are high, and not such a marked effect when air counts are low.

Day	Time		
	1	2	3
	% Decrease	% Decrease	% Decrease
1	84.1	80.2	41.3
2	7.8	38.4	84.2

Table 9. Percentage Decrease in Microbial Density by Day of week of Testing and Time of Testing.

Day	Time	1		2		3	
	Filter	Before (CFU/m ³)	After (CFU/m ³)	Before (CFU/m ³)	After (CFU/m ³)	Before (CFU/m ³)	After (CFU/m ³)
1		88	14	177	35	63	37
2		64	59	151	93	120	19

Table 10. Joint Effect on Microbial Density of Combination of Filter Placement, Day of Testing and Time of Testing.

Table 9 illustrates the percentage decrease in microbial density upon interaction of filter placement, day of testing and time of testing. Table 10 illustrates the variations in microbial density for another three-way combination of factors - where microbial density is influenced by the placement of the filtration system *and* the day of the week on which the tests were run *and* the time of testing. Overall a reduction in microbial density can again be observed, although the reduction in microbial density for the morning on Day 2 is not as marked as for Day 1.

Despite the variations in reductions of microbial density as highlighted by the Analysis of Variance, the global results of the study indicate that the filter has a statistically significant effect on microbial counts. Overall, the filter reduces the geometric mean airborne density by a factor of about 0.35.

Staff Questionnaire Results

Eight clinicians completed the questionnaires. All of these clinicians were female. Four respondents completed the questionnaire for clinic 1, with 2 respondents completing the questionnaire for clinics 2 and 3.

Seven of the respondents currently experience at least one of the symptoms associated with SBS (Table 11), with some of the clinicians experiencing up to four symptoms. These symptoms include ocular problems, headaches, dry/sore throats, coughing, sinus problems and allergies. The majority of these practitioners also experience health problems which make them susceptible to such symptoms. These include the wearing of contact lenses, allergies and asthma.

Number of Symptoms	Frequency
0	1
1	1
2	1
3	2
4	3

Table 11. Total Number of Symptoms Experienced by Clinicians.

Six of the respondents were aware of other podiatrists who were suffering from similar problems. Of those suffering symptoms 5/7 were able to state that the problems, which they

were experiencing, are exacerbated during working hours. The rest of the respondents stated that their symptoms were variable with no set pattern. Of the respondents who stated that the working environment aggravated their symptoms, these symptoms were noted to decrease at weekends and holiday times.

All of the respondents stated that they spent most of their working times in the podiatry treatment areas/ orthotic laboratories (as appropriate) and that this is where they experienced most discomfort with regard to their symptoms. Factors observed with regard to building conditions, which may help to explain some of the symptoms experienced, include a lack of ventilation, dust/dirt gathering around ventilation ducts and uncomfortable working temperatures i.e. too warm and humid.

Of the respondents suffering symptoms, 5/7 considered their symptoms to be serious enough to warrant medical attention and other clinicians had made conscious changes to their working patterns, which reduced their exposure times to dusts and fumes.

Upon installation of the filtration systems, 3/7 of the practitioners suffering symptoms felt that their problems rated 'about the same' in severity. One of the respondents stated that their symptoms were 'slightly better', with the same percentage stating that their symptoms were 'a lot better'. Two of the respondents stated that they thought that it was 'too early to tell' and that the filtration systems had not been in place long enough for them to comment about their efficiency or effectiveness. Six of the total number of respondents did however, notice a difference in the environment of the treatment rooms. These differences were described as perceptible differences to the air - being cooler, fresher, less 'stuffy' and less 'stagnant'.

Although 6 of the practitioners stated that the machines were noisy, generally, this noise was not to such an extent that it was disruptive or troublesome, except to 2 of the practitioners. Four practitioners stated that patients had commented on the excessive noise levels of the machines, but had simultaneously commented that the room was fresher and cooler.

When asked if they would wish such a unit to be made available to them, 5 of the practitioners stated that they would. Concerns among the other practitioners were again that the machines were noisy (except that this could be accommodated if it benefited the working environment), too large and obtrusive for the treatment areas, while others were undecided due to the short time frame of the study.

Conclusions

With regard to the qualitative data collected within this study, all of the practitioners, with the exception of one, experienced symptoms concurrent with the patterns of SBS. However, the filtration units in most cases did not appear to greatly influence the number or severity of the symptoms suffered. A few practitioners did, however, comment that they considered the time frame of the study to be too short to fully appreciate any benefits. It should be noted also that the number of respondents completing the questionnaires was small.

Many of the practitioners also commented upon the excessive noise of the filtration units, in most cases this was considered not to be to such a level that it became troublesome. Some of the practitioners also commented upon the cumbersome and obtrusive nature of the units, although this problem could be addressed by the installation of ceiling-mounted units. In view of the comments made regarding noise and size, however, the majority of practitioners stated that they would wish one of the filtration units to be installed in their clinic, if the option was made available to them.

With regard to the quantitative data collected within this study, the results clearly demonstrate a statistically significant reduction in the number of CFU/m³ upon installation of the filtration units by a factor of 0.35 (although this appears to be influenced by a number of factors such as the day and time and clinic under test). In addition to quantifiable measures, subjective data collected by the questionnaire suggests that the filtration units do improve the quality of the indoor air, with many of the respondents and some of the patients commenting that the room environment was much 'fresher' and 'cooler'.

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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**Department of PPR and Clean Air Ltd
Occupant Interview**

Page 1 of 2

Building Name: _____ File Number: _____

Address: _____

Occupant Name: _____ Work Location: _____

Completed by: _____ Title: _____

Date: _____

SYMPTOM PATTERNS

What kind of symptoms or discomfort did you experience before the Filter system was installed?

Are you aware of other people with similar symptoms or concerns? Yes No

If so, what are their names and locations?

Do you have any health conditions that may make you particularly susceptible to environmental problems?

Contact lenses	<input type="radio"/>	Chronic cardiovascular disease	<input type="radio"/>
Allergies	<input type="radio"/>	Asthma	<input type="radio"/>
Chronic respiratory disease	<input type="radio"/>	Immune system suppressed by disease	<input type="radio"/>
Other causes	<input type="radio"/>	Chronic neurological problems	<input type="radio"/>

TIMING PATTERNS

When did your symptoms start? _____

When are they generally worst? _____

Do they go away? If so, when? _____

Have you noticed any other events (such as weather events, temperature or humidity changes, or activities in the building) that tend to occur around the same time as your symptoms?

SPATIAL PATTERNS

Where are you when you experience symptoms or discomfort? _____

Where do you spend most of your time in the building? _____

ADDITIONAL INFORMATION

Do you have observation about building conditions that might need attention or might help explain your symptoms (e.g. temperature, humidity, drafts, stagnant air, odors)?

Have you sought medical attention for your symptoms? _____

Do you have any other comments? _____

SINCE AIR FILTER WAS INSTALLED

Are your symptoms:

A lot worse Slightly worse About the same Slightly better A lot better

Do you feel any difference in the room environment? _____

If yes, what is the difference? _____

Is the machine noisy? Yes No

If yes, does it bother you? Yes No

Have any patients commented on the equipment, atmosphere noise? Yes No

If yes, what comments have they made? _____

When did your symptoms start once the filter was in place? _____

When are they generally worse now that the filter is in place? _____

Do they go away? If so, when? _____

Would you wish one of these units in your clinic if you were given the opportunity? Yes No

Any other comments

Statistical Methodology

Air sampling was carried out at three **clinics** (1, 2 and 3). At each clinic, sampling was carried out on two **days** (Monday and Thursday) before **filter** units were in place, and on two days (Monday, Thursday of the following week) after filters units were in place. On each sampling day at each clinic, sampling was performed at three **times** of day (morning, mid-day and late afternoon). Air samples were obtained for two **positions** – face level (where the chiropodist was working), and away from the immediate working area (background sample).

Table 12 shows how the cross-classification of the five factors: Clinic, Time, Position, Filter, Day (of the week) gives rise to the 72 observations collected.

Clinic	Time	Position	Filter			
			Before		After	
			Day		Day	
			Monday	Thursday	Monday	Thursday
1	AM	Face				
		Background				
	Mid-day	Face				
		Background				
	PM	Face				
		Background				
2	AM	Face				
		Background				
	Mid-day	Face				
		Background				
	PM	Face				
		Background				
3	AM	Face				
		Background				
	Mid-day	Face				
		Background				
	PM	Face				
		Background				

Table 12. Cross-classification of the five factors: Clinic, Time, Position, Filter and Day.

Response variable

The response variable is the natural log of microbial density (per litre of air). 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 five factors (Clinic, Time, Position, Filter, 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 Clinic is 2. 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 5 factors whose influence is to be examined. Thus, potentially, an analysis of variance table could contain interactions including up to 5 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 and 5 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 4-factor interactions, and the single 5-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.

One small detail is that two of the microbial densities are equal to zero. 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.

$$\exp(\text{predicted mean}) - 1.0$$

These predicted means are approximately equal to geometric mean microbial densities.