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Particle and microbial airborne dispersion from people

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The airborne dispersion of particles from 55 people (30 females and 25 males) was measured. The dispersion per minute of microbe carrying particles (MCPs) averaged 2,400 when wearing personal indoor clothing, and 177 when wearing cleanroom garments. One exceptional person, whose dispersal rates were not included in these results, dispersed 11,000 per minute when wearing cleanroom garments. The dispersion rate of particles $\geq 5\mu\text{m}$ per minute averaged 332,000 when wearing indoor clothing, and 37,300 when wearing cleanroom garments. The dispersion rate of particles $\geq 0.5\mu\text{m}$ per minute averaged 2,130,000 when wearing indoor clothing, and 1,020,000 when wearing cleanroom garments. The dispersion rates for particles and MCPs were higher in males than females. Depending on the method used, the average equivalent particle diameter of the MCPs was $9\mu\text{m}$ or $18\mu\text{m}$.

There was no situation where the dispersion of MCPs was not accompanied by substantial numbers of both $\geq 0.5\mu\text{m}$ and $\geq 5.0\mu\text{m}$ airborne particles, and there appears to be little advantage in measuring particles $\geq 5.0\mu\text{m}$ when using airborne particle counting to indirectly monitor the dispersion of MCPs. When wearing cleanroom garments, the ratio of $\geq 0.5\mu\text{m}$ particles to MCPs was found to average 5,800:1, and for $\geq 5.0\mu\text{m}$ particles it was 210:1.

Key words: Particle; microbial, airborne, dispersion, people

Introduction

Microbes in cleanroom air are almost exclusively derived from personnel within the room. People shed one outermost layer of epithelial cells every 24 hours, this amounting to about 10^9 cells per day¹. Skin cells are approximately $33\mu\text{m} \times 44\mu\text{m}$ in surface area and about 3 to $5\mu\text{m}$ thick, and may be found in the environment either as whole cells or fragments, the median size across the surface being about $20\mu\text{m}$, with 7-10% less than $10\mu\text{m}$ ¹. Micro-organisms grow on the cells and glands of the skin and are dispersed into the air on skin detritus. These particles are therefore often called microbe carrying particles (MCPs). MCPs will vary in size, shape, and density, and it is conventional to consider the size of particles in terms of equivalent particle diameter, which is the size of a sphere of unit density that has the same aerodynamic properties as the particle being considered. It therefore follows that a skin cell, which has a surface diameter of about $20\mu\text{m}$ but a thickness of 3 to $5\mu\text{m}$ is likely to have an equivalent particle diameter below $20\mu\text{m}$. Noble, Lidwell and Kingston², and Whyte³, have investigated the equivalent particle diameter of MCPs dispersed from people, and Whyte, Green and Albus⁴ have compiled these results and reported that the average size is about $12\mu\text{m}$, with 1% below $1\mu\text{m}$, 25% below $7\mu\text{m}$, 25% above $24\mu\text{m}$, and 5% above $50\mu\text{m}$. This size distribution

shows that if non viable particles $\geq 0.5\mu\text{m}$ were sampled by use of a particle counter then 100% of MCPs would be counted and, if particles $\geq 5.0\mu\text{m}$ were sampled, it can be calculated using the method given in Whyte, Green and Albus's paper that about 83% of the MCPs would be counted.

MCPs and inert particles are kept at a low concentration in cleanroom air. This is done by supplying HEPA filtered air and by minimising the dispersion from people by use of occlusive clothing. From a knowledge of the dispersion rate of MCPs and particles, as well as the air supply to a room, the likely airborne concentration in a cleanroom can be calculated, this method being described by Whyte⁵, and Ljungqvist and Reinmüller⁶.

The reduction of the dispersion of MCPs by cleanroom garments has been reported by Whyte and Bailey⁷, who have also reported on the reduction of the dispersion of particles⁸. Reinmüller and Ljungqvist⁹ have also published information on this topic. However, these and similar dispersion studies have been carried out on one person, or on small groups of people. Additional information is required to establish the rates of dispersal of MCPs and total particles by the normal population, and on the ratio of total particles to MCPs, and differences in dispersion rates between males and females.

Annex 1 (2003) of the European Commission Guide to Good Manufacturing Practice (EC GGMP)¹⁰ gives information on concentrations of airborne particles and microbes that should not be exceeded in cleanrooms where sterile pharmaceutical products are manufactured. Airborne MCPs

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must be controlled in cleanrooms, or they may deposit into pharmaceutical products. However, the requirement for controlling inert airborne particles is less clear. Although an occasional inert particle will deposit from the air into containers, the particle deposition into containers has been studied in several pharmaceutical manufacturing cleanrooms, and shown to be insignificant with respect to the regulatory limits set^{11, 12}, and unlikely to harm patients who receive the product¹³. Particles found in containers after manufacture do not come from air but from containers and stoppers¹¹. It is even less clear why airborne particles $\geq 5\mu\text{m}$ should be measured in pharmaceutical cleanrooms, especially as their concentration is so low as to be below accurate and reliable measurement. The FDA Guidance (2004)¹⁴ does not have such a requirement. Informal discussions with the European Medicines Agency have suggested that they consider that because particles $\geq 5\mu\text{m}$ are closer in size to MCPs than $\geq 0.5\mu\text{m}$ particles, they need to be monitored. It has also been suggested that particles $\geq 5\mu\text{m}$ can be found with no accompanying particles $\geq 0.5\mu\text{m}$, and therefore need to be independently counted. These suggestions require investigation.

Measurement of particle and microbial dispersion from people

The measurement of airborne particles and microbe carrying particles was carried out in a dispersal chamber. This is the same design as was used in a series of studies starting in 1976¹⁵. It has the advantage over previous designs as the airborne dispersion rate, i.e. number per minute, can be measured.

Description of dispersal chamber

The dispersal chamber where a person exercised is shown in **Figure 1**. It was 0.68m x 0.52m x 2m high and was made of metal frame covered with clear plastic sheet. Air was supplied at the top of the chamber and passed through a HEPA filter (a) into the chamber. Air was normally supplied at just over 700L/min, and balanced by the removal of air by a high-volume bacterial sampler (Casella slit sampler) operating at 700L/min, and an airborne particle counter operating at 2.83L/min, at sampling port (c). A slight positive pressure was maintained inside the chamber to ensure that no contamination entered the chamber from outside. This outward flow was checked by an anemometer at the exhaust ports (d).

Personnel tested and clothing worn

Testing was carried out on 55 people (30 females and 25 males). All of the females worked within a cleanroom of a pharmaceutical manufacturing company. So did the majority of men, but to make the numbers more even, some male technicians from Glasgow University were tested. Each subject was tested while wearing the following two types of clothing:

- 1 Personal indoor clothing: Trousers were worn by all subjects except 6 females, who wore skirts. A blouse, shirt, or T shirt was also worn, and clean plastic overshoes were worn over shoes.

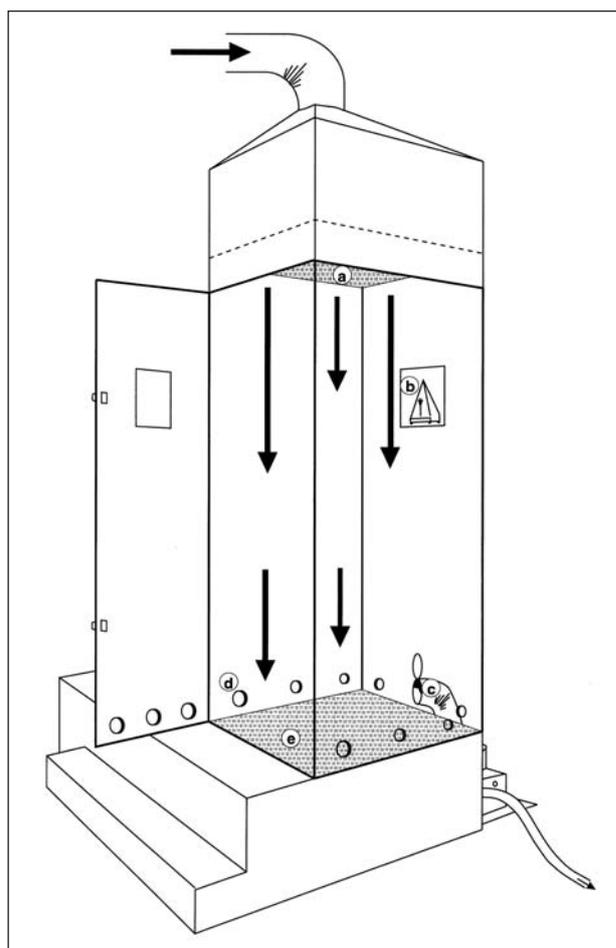


Figure 1. Dispersal chamber.

- 2 Cleanroom garments, manufactured from woven polyester fabric, were worn on top of the person's indoor clothing. It has been shown that the dispersion rate is dependant on whether the cleanroom clothing is new, or washed and sterilised⁹. The garments were supplied by the pharmaceutical company and it was not clear how often they had been worn. It was certain that none of the garments were new, and the average use was much greater than 50 times. The polyester fabric was tested for pore diameter according to the method laid down in the IEST Recommended Practice 003.3¹⁶. This test is a good indicator of the filtration and occlusive properties of the fabric, which was shown to have a pore diameter of $25\mu\text{m}$. A coverall was worn and its trouser bottoms were covered by knee-length boots. The subject also wore disposable latex gloves, disposable mask and woven polyester hood, the hood being tucked into the neck of the coverall, leaving only the eye area uncovered.

Dispersal chamber test procedure

The test procedure was as follows:

- 1 The steps leading into the chamber were disinfected.
- 2 The particle counter, which was used to count particles $\geq 0.5\mu\text{m}$ and $\geq 5.0\mu\text{m}$, was switched on and the fan speed increased to its maximum to flush the chamber and reduce the airborne particle count to practically zero.

- 3 The subject, wearing the required clothing entered the chamber. The door was then closed. The floor of the chamber was covered with a foam mat (e) dipped in disinfectant and wrung out. This ensured that a minimal amount of particle and microbial contamination was re-dispersed from the floor during exercise.
- 4 The subject stood at rest until the total particle count reduced to practically zero, and became steady.
- 5 The air supply was then reduced to just over 700L/min.
- 6 The subject started marching to the beat of a metronome (1 beat/s), while swinging one arm, and then the other, up to their shoulder. The metronome was visible to the person, its position (b) being shown in **Figure 1**.
- 7 After the first minute of the exercise, the bacterial air sampler, which had a sampling rate of 700L/min, was switched on and off for between 30s and 4 min, depending on the likely dispersion rate. Males wearing their personal indoor clothing were tested for 30s, and when wearing cleanroom clothing they were tested for 2 minutes. Females wearing their personal indoor clothing were tested for 1 minute, and for 4 minutes when wearing cleanroom clothing.
- 8 MCPs were deposited onto plates containing tryptone soya agar (Oxoid Ltd) supplemented with 0.5% polysorbate 80 to aid the growth of lypophylic skin bacteria. The plates were incubated aerobically for 48 hrs at 37°C before counting the bacterial colonies. To minimise errors, plates were incubated before use and checked for sterility.
- 9 The particle counter simultaneously recorded the concentrations of the total particles greater, or equal to, 0.5µm and 5.0µm during the exercise.
- 10 After exercising, the person left the chamber, and the air supply was increased to a maximum to flush the chamber.

Calculation of the dispersion rate

The chamber was designed to give a downflow of unidirectional air. This ensured that during exercise the concentration of airborne contamination came quickly up to a maximum plateau concentration. Tests were carried out that established the airborne contamination took one minute to reach a maximum. This was used as the time when microbial and particle sampling began. Assuming the concentration of airborne dispersion reached a steady state i.e. the dispersion of particles is balanced by their removal, the dispersion of airborne contamination per minute can be calculated by use of the following equation:

$$D = C \times Q/S$$

Where,

D = Total number of MCPs, or particles, dispersed per minute

C = Total number of airborne contaminants measured by the microbial sampler, or particle counter, per minute

Q = air supply rate to chamber (700L/min)

S = sampling rate of microbial sampler (700L/min), or particle counter (2.8L/min)

In the case of the MCPs, the volume of the air supplied to, and removed from, the chamber by the slit sampler can be assumed to be the same, and hence the equation simplifies to the following:

$$D = C$$

In the case of the particle sampler, the sampling rate was 2.83L/min, and the particle dispersion per minute calculated as follows:

$$D = C \times 700/2.8$$

Sampling efficiency of the microbial sampling method

As can be seen in **Figure 1**, the airborne sampler was connected at the bottom of the chamber by a 10cm diameter duct. Because of the height of the sampler's intake and the distance of the chamber from the floor, the duct had to turn through 90° in a short distance. The duct's sharp turn was likely to cause losses due to impaction of the MCPs. Some preliminary tests were carried out to ascertain these losses, and they were thought to be about 25% of the total. It was also known that some microbial samplers have low collection efficiency¹⁷, and therefore some collection losses were likely when using the Casella slit sampler. When tested in comparison to other samplers, the Andersen sampler is normally found to be the most efficient sampler available¹⁷, especially when its entrance cone is removed¹⁸ and it has been suggested as the sampler by which the efficiency of other samplers should be assessed^{19,20}. However, the Casella sampler was chosen in preference to the Anderson sampler because of its much higher air sampling rate, which enabled low dispersion rates to be measured.

To ascertain the combined losses from the intake duct and the air sampler, a series of experiments using 20 people (11 females and 9 males) compared the counts from two Anderson samplers (without their entrance cones) placed on the floor of the dispersal chamber, with the attached Casella slit sampler. Indoor clothing was worn and the average count obtained from the slit sampler was 524/min, and 1366/min from the Andersen sampler. This gives a ratio between the two counts of 2.6:1, and this ratio was used to recalculate the count obtained from the slit sampler i.e. the counts from the slit sampler were multiplied by 2.6.

Results of particle and microbial dispersion in chamber

All of the results in this section are given to 3 significant places. The results given in this section do not include the exceptionally high dispersion rate of MCPs obtained from one person. This is discussed in the next section of this paper.

Shown in **Figure 2** is the dispersion rate per minute of MCPs, particles ≥0.5µm, and ≥5µm, obtained from 55 people wearing cleanroom garments. The counts on the left are from females, and those on the right are males.

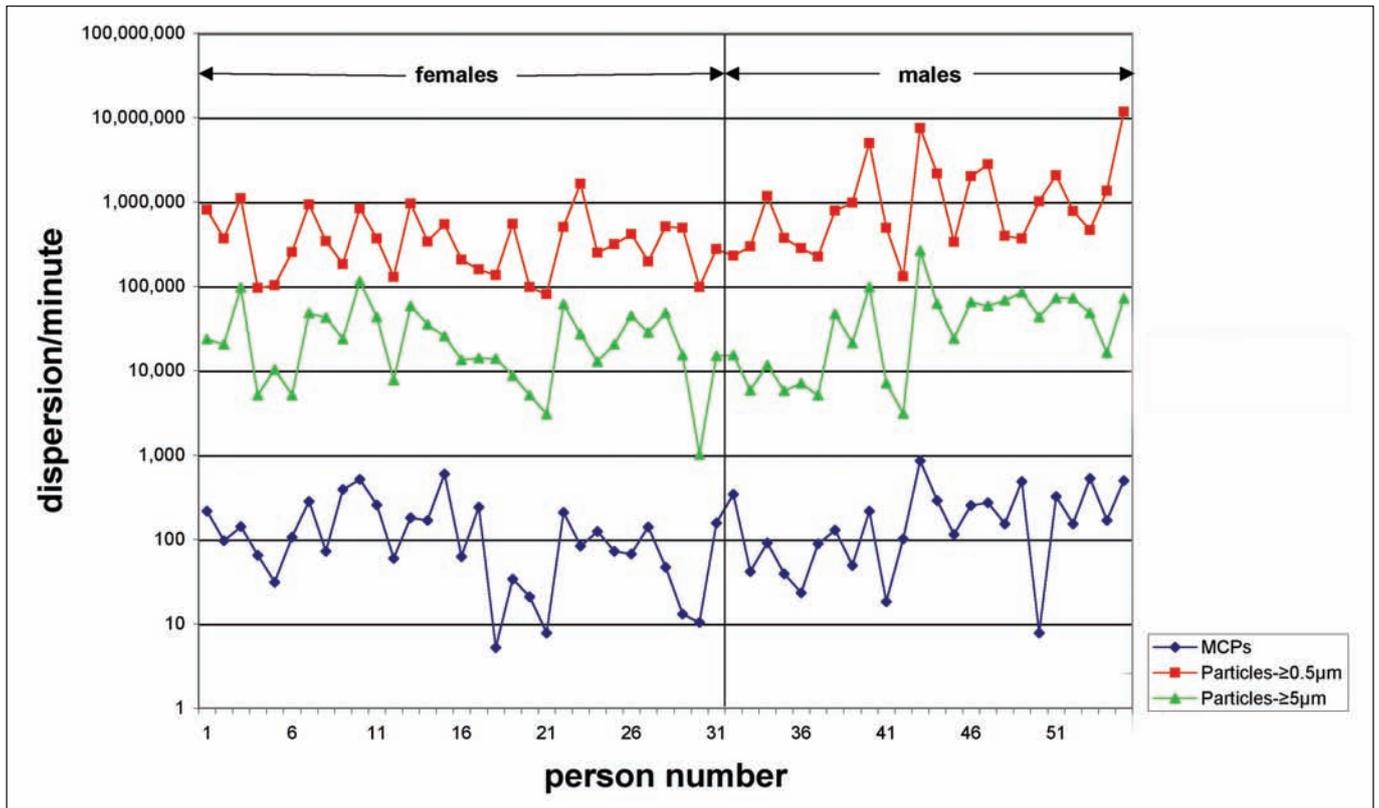


Figure 2. Dispersion of MCPs and particles from 55 people wearing cleanroom garments. Dispersal rates are arranged to show the rates from 30 females on the left and 25 males on the right.

Shown in Figure 3 is a plot of both the number of particles $\geq 0.5\mu\text{m}$ and $\geq 5\mu\text{m}$ dispersed per minute from the 55 people in comparison to the dispersal rate of MCPs from the same people.

Given in Table 1 are the average and range of the dispersion of MCPs per minute. These results are also given for males and females wearing both their normal indoor clothing and cleanroom garments.

Given in Table 2 are the average and range of counts of the dispersion per minute of particles $\geq 0.5\mu\text{m}$ and $\geq 5\mu\text{m}$.

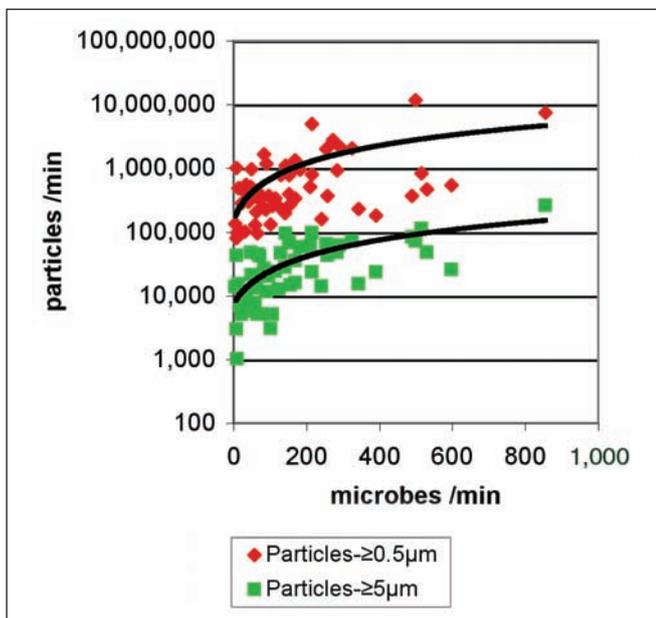


Figure 3. Relation of MCPs to $\geq 0.5\mu\text{m}$ and $\geq 5\mu\text{m}$ particles.

These results are also given for males and females wearing both indoor and cleanroom garments.

Given in Table 3 is the reduction in airborne dispersion of MCPs and particles when people wore cleanroom garments over their indoor clothing.

Given in Table 4 are the ratios of the number of particles and MCPs dispersed per minute by males compared to females. The males always gave a higher dispersion of particles. A two-sample t test was used to compare the counts obtained from the males and females. The dispersion rates of MCPs, particles $\geq 0.5\mu\text{m}$, and particles $\geq 5\mu\text{m}$ were compared between males and females when they wore both cleanroom and indoor personnel clothing. The statistical analysis showed this difference, in all comparisons, to be highly significant i.e. $P < 0.001$.

Given in Table 5 are the ratios of the number of particles $\geq 0.5\mu\text{m}$ and $\geq 5\mu\text{m}$ dispersed per minute, compared to the number of MCPs dispersed per minute.

Table 1. Average number and range of the dispersion rate per minute of MCPs from 55 people (25 males and 30 females)

	Indoor clothing – MCPs	Cleanroom garments – MCPs
All - averages	2,400	177
All –range	94 to 13,800	5 to 855
Females - averages	943	144
Females- range	94 to 6,630	5 to 514
Males - averages	4,160	216
Males -range	109 to 13,800	7.8 to 855

Table 2. Average number and range of the dispersion rate per minute of $\geq 0.5\mu\text{m}$ and $\geq 5\mu\text{m}$ particles from 55 people (25 males and 30 females).

	Indoor clothing		Cleanroom garments	
	particles $\geq 0.5\mu\text{m}$	particles $\geq 5\mu\text{m}$	particles $\geq 0.5\mu\text{m}$	particles $\geq 5\mu\text{m}$
All – averages	2,130,000	332,000	1,020,000	37,300
All – range	142,000–14,500,000	3,810-2,110,000	79,700-11,700,000	1,020-263,000
Females – averages	1,720,000	257,000	432,000	29,000
Females – range	142,000-11,800,000	3,810-2,110,000	79,700-1,640,000	1,020-114,000
Males – averages	2,630,000	422,000	1,720,000	47,300
Males – range	250,000-14,500,000	6,350-1,680,000	222,000-11,700,000	3,050-263,000

Table 3. Number of times reduction in airborne dispersion when cleanroom garments were worn over personnel indoor clothing

	MCPs	Particles $\geq 5.0\mu\text{m}$	Particles $\geq 0.5\mu\text{m}$
All	13.6	8.9	2.1
Females	6.5	8.9	4
Males	19.3	8.9	1.5

Table 5. Ratios of the number of particles $\geq 0.5\mu\text{m}$ and $\geq 5\mu\text{m}$ dispersed per minute compared to the MCPs

Indoor clothing		Cleanroom garments	
particles $\geq 0.5\mu\text{m}$	particles $\geq 5\mu\text{m}$	particles $\geq 0.5\mu\text{m}$	particles $\geq 5\mu\text{m}$
890:1	140:1	5,800:1	210:1

Table 4. Ratios, males: females of the number of particles and MCPs dispersed per minute.

Indoor clothing			Cleanroom garments		
MCPs	$\geq 0.5\mu\text{m}$	$\geq 5\mu\text{m}$	MCPs	$\geq 0.5\mu\text{m}$	$\geq 5\mu\text{m}$
4.4:1	1.5:1	1.6:1	1.5:1	4.0:1	1.4:1

Table 6. Number of MCPs dispersed per minute from two male personnel wearing indoor and cleanroom garments.

Person tested	Indoor clothing	Cleanroom garments
Prolific disperser	16,100	11,000
Control person	1,590	6

Microbial dispersion from a prolific disperser

A dispersal chamber test was carried out on one unusual individual. This was in addition to the 55 people, and only microbial dispersion was recorded. The individual had been working for some time in a cleanroom without incident. However, an unusually high percentage of microbes in the room quite suddenly appeared and were identified as *Micrococcus luteus*. The microbial flora of the skin of this person had changed so that it had become predominately *Micrococcus luteus*, and gave an uncountable concentration on the contact plate samples taken from his clothing. The cause of this change was uncertain but he had received antibiotic treatment which could have changed his skin flora. He was withdrawn from the cleanroom and tests carried out in the dispersal chamber.

Tests were carried out on the dispersion of MCPs using the method described in the previous section. These were carried out on both the person and another male, who acted as a control. Both were tested using their indoor clothing i.e. shirt and trousers, and then with cleanroom garments. The cleanroom garments consisted of sterile factory trousers and shirt, with a sterile polyester coverall on top. The coverall, hood, and full length boots were made of the same fabric and design as used in the previous set of tests carried out on 55 people. Disposable mask, polyester hood, and sterile disposable latex gloves were also worn.

Given in **Table 6** is the microbial dispersion rate obtained from the prolific disperser and the control person, when wearing personal indoor clothing and then cleanroom garments. The MCPs sampled from the prolific disperser were almost exclusively one species i.e.

Micrococcus luteus, which was most unusual. Two tests were carried out for each clothing condition, and these were averaged. The counts were then normalised by multiplying them by 2.6 to take account of the efficiency of the microbial air sampler, as previously determined.

The control person gave microbial dispersion rates within the range of values found with the tests carried out on 55 people, but the prolific disperser gave unusually high ones. Shown in **Figure 4** is the microbial dispersion

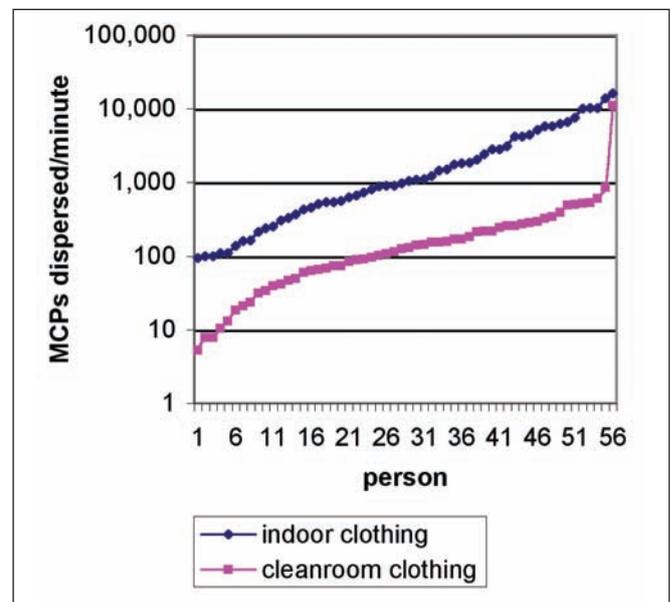


Figure 4. Dispersion of MCPs per minute from 55 people and the additional prolific disperser when wearing indoor and cleanroom garments.

rates obtained from the 55 people when wearing either indoor or cleanroom garments, and arranged in order so that the lowest counts are on the left and the highest counts are on the right. Also added to the right hand side of the graph are the counts obtained from the prolific disperser (person number 56). The maximum dispersion rate previously observed from the 55 people studied, when wearing indoor clothing, was 13,800 per minute, and when wearing cleanroom garments was 855 per minute. The prolific disperser shed considerably more than the previously highest disperser when wearing indoor clothing, and was 13 times more prolific than the previously highest disperser when wearing cleanroom garments.

Particle size of the microbe carrying particles dispersed

Calculated by an Andersen sampler

An Andersen sampler is a cascade microbial air sampler that has 6-stages, each stage having 400 holes which decrease in diameter down through the stages. The impaction velocity onto each agar surface therefore increases down through the stages and, as the air passes down through the stages, the size of particle that is efficiently deposited onto the agar plate becomes smaller. This allows the size distribution of the MCPs to be calculated. The percent cumulated counts, by Andersen stages, were calculated on a "less than stated size" for each sampler stage and plotted on log 3-cycle x probability paper, setting the plot point, in micrometres, as the 50% of the next stage above. The values for 50% cumulative particle size impacted on each stage, in terms of equivalent particle diameter, were obtained from published results²¹. These were as follows:

Stage 1 = 9.8 μ m
 Stage 2 = 6.2 μ m
 Stage 3 = 3.8 μ m
 Stage 4 = 2.2 μ m
 Stage 5 = 0.9 μ m

The method is more fully explained elsewhere³. From the line of best fit, the equivalent median diameter is read from the graph opposite the 50% probability. The result obtained of the median equivalent particle diameter when wearing cleanroom garments was 9 μ m.

Calculated by deposition velocity

The average deposition velocity of MCPs can be calculated from the following equation.

$$\text{Deposition velocity (m/s)} = \frac{\text{Number settling/m}^2/\text{s}}{\text{Number in air/m}^3}$$

The numbers of MCPs settling/m²/s onto a surface can be obtained from settle plates and the number/m³ in the cleanroom air from an air sampler. To obtain this information, 6 plates, each of 14cm diameter (154cm² surface area) were arranged around the person exercising on the dispersal chamber floor, giving a total surface area

of 0.924m². These plates were exposed for 120 seconds and an average count of 220 was obtained from 20 people who were sampled.

Therefore,

$$\text{Number settling on plates/m}^2/\text{s} = 220 \times 1/0.94 \times 1/120 = 1.984$$

At the same time as the plates were exposed, an Andersen sampler (without entrance cone) was placed on the floor of the chamber and used to measure the MCPs in the air. This gave an average count of 1.951/m³.

Therefore,

$$\text{Deposition velocity (m/s)} = 1.984 \div 1.951 = 1.02 \text{ cm/s}$$

Because of their comparatively large size, MCPs deposit onto surfaces under the influence of gravity³. Based on Stokes Law, the following equation can be derived²² that relates the settling velocity of airborne particles in air to the equivalent particle diameter:

$$\text{Settling Velocity} = \frac{\rho d^2 g}{18\eta}$$

where,

ρ = density of particle

d = equivalent diameter of particle.

g = acceleration due to gravity

η = viscosity of air

This equation applies to spherical objects. As has been explained in the introduction of this paper, MCPs are not spherical, but it is conventional to consider airborne particles in terms of equivalent particle diameter, which is the size of a sphere of unit density that has the same aerodynamic properties as the particle being considered. Using this approach the density of a particle in the above equation can be taken as unity (1g/cm³). The viscosity of air at 20°C is 1.84 x 10⁻⁵kg/m.s, and if the particle diameter units is expressed in μ m, then:

$$\text{Settling velocity (cm/s)} = 0.0032 d^2$$

$$\text{or, } d = \sqrt{\frac{\text{settling velocity (cm/s)}}{0.0032}}$$

In our example:

$$\text{Equivalent diameter of MCPs} = \sqrt{\frac{1.02}{0.0032}} = 18\mu\text{m}$$

Discussion and conclusions

The dispersion rate of MCPs, particles $\geq 0.5\mu$ m, and $\geq 5.0\mu$ m from 55 people was measured. Thirty females and 25 males were studied, this being the largest study that the authors are aware of. The dispersion rates given in this paper can therefore be used with more confidence when such information is required, for example, to calculate the expected airborne contamination concentration in cleanrooms.

Large variations in airborne dispersion from people were apparent, and the rates of dispersion of MCPs, particles $\geq 0.5\mu\text{m}$, and $\geq 5.0\mu\text{m}$ were interlinked i.e. high dispersers of one type of particle would disperse high rates of particles of the other two types, and vice versa. The number of MCPs dispersed per minute, when wearing indoor clothing, and excluding one exceptional individual, ranged from 94 to 13,800 (average = 2,400), and when wearing cleanroom garments ranged from 5 to 855 (average=177). One exceptional individual dispersed 16,000 MCPs per minute when wearing indoor clothing, and 11,000 per minute when wearing cleanroom garments. The dispersion rate per minute of airborne particles $\geq 0.5\mu\text{m}$ ranged from 142,000 to 14,500,000 (average=2,133,000) when wearing indoor clothing, and from 79,700 to 11,700,000 (average=1,020,000) when wearing cleanroom garments. Similarly, the dispersion rate of airborne particles $\geq 5.0\mu\text{m}$ ranged from 3,810 to 2,110,000 (average=332,000) when wearing indoor clothing, and from 1,020 to 263,000 (average= 37,300) when wearing cleanroom garments.

The reduction in the dispersion rates, when cleanroom garments were worn over personal indoor clothing, was 13.6-fold for MCPs, 8.9-fold for particles $\geq 5.0\mu\text{m}$, and 2.1-fold for particles $\geq 0.5\mu\text{m}$. It has been previously demonstrated^{7,8} that the reduction in the dispersion of MCPs and particles by the use of cleanroom garments was determined by the tightness of the weave of the cloth, and the design of the garments. The more occlusive the fabric and garments, the greater the reduction in airborne dispersion, and more larger particles would be retained than smaller particles. The effectiveness of the cleanroom garments studied in this study was therefore as expected.

The relative dispersion from males and females was studied. It was found that men dispersed greater numbers of MCPs and particles and this was confirmed by statistical analysis. It is well established that males disperse more MCPs than females²³, but the authors are not aware of any study that has established that males also disperse more inert particles. It has been suggested by Noble^{23,24} that the possible reasons for the higher rates of dispersion of MCPs by males is because of the higher concentration of bacteria on their skin, slightly smaller size of skin cell, different rates of dispersion from different regions of the body, and a greater area of skin. However, McIntosh *et al*¹ have suggested that differences need only be explained by the higher concentration of bacteria on the skin of males. Not all skin cells are colonised by bacteria, and an increase in bacterial concentration results in more skin cells being colonised, and hence a greater dispersion rate of MCPs. This latter explanation would fit better with an unpublished study we carried out, where we failed to show that the size of MCPs dispersed from males was smaller than females, or that the weight and size of an individual (which should reflect their skin area) influenced the dispersion rate. However, the full explanation for the differences is uncertain.

The equivalent particle size of MCPs was calculated using two methods. An Anderson sampler gave an equivalent particle diameter of 9μ , and a deposition

velocity method, which used simultaneous sampling by settle plates and an Anderson sampler, gave an average size of $18\mu\text{m}$. The Andersen sampler is possibly the most efficient sampler commercially available, but has losses in the intake to the sampler, which selectively remove larger particle sizes¹⁸. This loss was minimised by not using the intake cone, but deposition losses on the intake holes on the top stage will reduce the average particle diameter. There is also some doubt as to whether the d_{50} sizes used to calculate the average equivalent diameter are accurate, especially the values used for the top stages¹⁸. When using the settling velocity method, reliance can be placed on the settle plate correctly measuring the settling rate, but the Andersen sampler may underestimate the concentration of MCPs in the air. The correct average equivalent diameter is therefore likely to be between the values produced by the two methods, and the previously suggested size of $12\mu\text{m}$ ⁴ still appears a reasonable value.

The ratios of the number of particles dispersed of $\geq 0.5\mu\text{m}$ and $\geq 5.0\mu\text{m}$ diameter, to the number of MCPs, when wearing cleanroom garments was 5,800 and 210, respectively. These ratios are similar to those reported by Reinmüller and Ljungqvist⁹, who found that the $\geq 0.5\mu\text{m}$ particles to MCPs ratios were between 1,500:1 and 8,000:1, and for the $\geq 5.0\mu\text{m}$ particles to MCPs were between 24:1 and 140:1. These ratios will vary depending on the volunteers, the design of the clothing, the type of clothing fabric, and whether the fabric was new, unwashed and unsterilised. Changes in the ratios may also occur owing to differences in the sampling efficiency of microbial air samplers, and different losses in the intake methods of the particle counters and microbial samplers. The ratios are also likely to be greater in cleanrooms if there are additional particle dispersing mechanisms other than from people e.g. from machinery.

In Annex 1 (2003) of the EC GGMP¹⁰, upper limits of MCPs and particle concentrations are given for pharmaceutical cleanrooms. Grade A areas should not exceed a concentration of $3,500/\text{m}^3$ for particles $\geq 0.5\mu\text{m}$, $1/\text{m}^3$ for particles $\geq 5.0\mu\text{m}$, and $1/\text{m}^3$ for MCPs. If it may be assumed that the concentration of MCPs ($1/\text{m}^3$) is the prime value that should not be exceeded in order to obtain a suitable quality of product, and that monitoring of particles gives additional indirect monitoring of MCPs, then the maximum count of $3,500/\text{m}^3$ for particles $\geq 0.5\mu\text{m}$ is reasonably close to that expected from the ratios found in this paper and by Reinmüller and Ljungqvist⁹. However, Annex 1 of the EC GGMP requires a count of 1 particle $\geq 5.0\mu\text{m}/\text{m}^3$ when the microbial limit is $1/\text{m}^3$. This microbial limit is too low to fit in with the ratio, and that suggested in ISO 14698-1 (29 particles $\geq 5.0\mu\text{m}/\text{m}^3$) is closer, although still low. It is also interesting to consider the ratios of particles $\geq 5.0\mu\text{m}$ to particles $\geq 0.5\mu\text{m}$. In this series, the ratio when wearing cleanroom clothing was found to be 27:1. Eaton²⁵ found an average ratio of 12:1 and 57:1 when sampling in his Grade A and Grade B cleanroom areas. In ISO 14644-1 the classification limits for $\geq 0.5\mu\text{m}$ particles are 121 times that of the $\geq 5.0\mu\text{m}$ particles e.g. in a Class 5 room the concentration of particles $\geq 0.5\mu\text{m}$ is $3,520/\text{m}^3$ and that of $\geq 5.0\mu\text{m}$ is 29.

However, in Annex 1 the requirement for a Grade A area is 3,500/m³ for particles $\geq 0.5\mu\text{m}$, but only 1/m³ for particles $\geq 5.0\mu\text{m}$; this requirement appears to be out of step with the normal ratio, and incorrect.

It has been suggested in justification of the requirement to count particles $\geq 5.0\mu\text{m}$ in pharmaceutical rooms that an occasional $\geq 5.0\mu\text{m}$ particle can be found in the air without the accompaniment of $\geq 0.5\mu\text{m}$ particles. It is quite clear from these present studies that MCPs are not dispersed by people without the accompaniment of both $\geq 5.0\mu\text{m}$ and $\geq 0.5\mu\text{m}$ particles, and these are in proportion to the number of MCPs dispersed. MCPs were never found without particles, and more particles $\geq 0.5\mu\text{m}$ are associated with microbial dispersion than particles $\geq 5.0\mu\text{m}$. Because of the distribution of the sizes of MCPs in cleanroom air, 100% would be measured when counting particles $\geq 0.5\mu\text{m}$, and from the known size distribution of MCP it can be ascertained that 83% of the MCPs would be measured when counting particles $\geq 5.0\mu\text{m}$. It therefore appears that the additional measurement of particles $\geq 5.0\mu\text{m}$ to ensure that MCPs are counted is unnecessary.

To ensure that a pharmaceutical product is fit for use by a patient, MCPs must be measured and controlled, as the deposition of airborne microbes is a major source of product contamination²⁶. However, the case for measuring and controlling particle concentration is less convincing. It has been demonstrated that very large changes in the airborne particle count had no measurable effect on the particle quality of the product, but that particle contamination came from the container and closures^{12, 13}. Similar conclusions were drawn from a series of experiments carried out by Dutch workers²⁷⁻²⁹. Particles must occasionally fall into the product but, in comparison to the levels of particles permitted in parenterals, it is insignificant^{11, 12}, and unlikely to be a problem to patients who are subsequently administered the product¹³. However, although the measurement of airborne particles is unnecessary to ensure the correct product quality, the measurement of particles is necessary to check that the cleanroom is functioning correctly. In this situation there is no benefit in measuring particles $\geq 5.0\mu\text{m}$, and because of the low concentration, and hence the reliability of the count obtained, as well as the long sampling time required because of the low concentration, it is a count to be avoided.

Particles $\geq 5.0\mu\text{m}$ are found in very low concentrations in pharmaceutical cleanrooms. To reliably measure them, very large sample volumes and sampling times must be used, and spurious results have a disproportionate effect. Eaton²⁵ has demonstrated this, and other practical difficulties associated with sampling this size of particle. It may be concluded that the measurement of particles $\geq 5.0\mu\text{m}$ in pharmaceutical cleanrooms is inaccurate and unnecessary. As the FDA do not require the measurement of particles $\geq 5.0\mu\text{m}$, the findings in this paper, if accepted by the European Medicines Agency would assist in the harmonization of the regulatory standards of the USA and the EU.

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