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Airborne microbial monitoring in an operational cleanroom using an instantaneous detection system and high efficiency microbiological samplers

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The airborne microbial contamination in a non-unidirectional airflow cleanroom, occupied by personnel wearing either full cleanroom attire or only cleanroom undergarments was simultaneously determined using an instantaneous microbial detection (IMD) system and efficient microbial air samplers that detected both aerobic and anaerobic microbes. Depending on the type of cleanroom clothing, the IMD system recorded between 7 to 94 times more 'biological' particles than microbe-carrying particles (MCPs) recovered by the air samplers. Change in the airborne concentration of 'biological' particles due to the different clothing was not consistent with the change in the concentration of MCPs. The median size of the 'biological' particles was smaller than the MCPs and the associated particle size distributions were considerably different. A number of sterile materials in the cleanroom were shown to disperse substantial quantities of 'biological' particles and it was concluded that the number of particles of microbiological origin, and the relationship between the counts of 'biological' particles to MCPs, were masked by non-microbial fluorescent particles dispersed from these materials. Consequently, adequate monitoring of this type of cleanroom operation to confirm appropriate airborne microbiological contamination control, using only an IMD system of the type used for this programme of work, is considered to be unfeasible. However, if the IMD system could be improved to more accurately differentiate between micro-organisms and non-microbial fluorescent particles, or if the dispersion of fluorescent particles from non-microbiological cleanroom materials could be reduced, then this system should provide an effective cleanroom airborne monitoring method.

Key words: Cleanroom, instantaneous microbial detection, Andersen sampler, airborne microbial contamination.

Introduction

Airborne microbial concentrations within pharmaceutical cleanrooms are traditionally determined by sampling a known volume of the cleanroom air and depositing, by impaction, the microbe-carrying particles (MCPs) onto nutrient media. The nutrient agar plates are incubated for several days and, under favourable conditions, the deposited micro-organisms will proliferate on the media to produce a visible cluster known as a colony. The number of colonies is counted to provide an estimate of the number of micro-organisms present in the air sample.

The IMD system utilised for this investigation draws in room air, which passes through a laser beam and two types of particles are measured and counted. Any particles present in

the air sample will scatter the laser light and the size of the particle is determined by measuring the quantity of the scattered light. These particles are known as 'total' particles in this article. Additionally, another optical sensor detects fluorescence, induced by visible laser light with a wavelength of 405nm, from any of the sized particles and also records them as 'biological' particles. The fluorescence produced from riboflavin (an essential constituent of all living cells), nicotinamide adenine dinucleotide (NADH; a coenzyme found in all living cells) and dipicolinic acid (a chemical compound of bacterial spores) can be detected by this IMD system. The detection of a full range of micro-organisms, including both viable and non-viable, aerobic and anaerobic, and those that do not grow using normal growth methods, is potentially possible with this technology. Using this system to automatically measure MCPs has a number of potential advantages for pharmaceutical cleanroom operations, particularly if immediate action can be initiated when defined limits have been exceeded, and significant resource savings

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are possible if traditional microbial air sampling need not be used. The use of IMD systems in cleanrooms has been discussed by Ljungqvist and Reinmüller¹ and Sandle².

To further investigate if an IMD system could be utilised for routine monitoring in operational cleanrooms, an IMD unit was compared with traditional, high collection efficiency types of airborne microbial samplers, and standard particle counters. This programme of work was undertaken in a cleanroom in one of the aseptic manufacturing facilities at AstraZeneca, Macclesfield (UK).

Cleanroom and test equipment

Cleanroom

The cleanroom in which the experiments were carried out was an EU GGMP Grade B³ pharmaceutical non-unidirectional airflow cleanroom, in which are located two EU GGMP Grade A³ unidirectional airflow containment cabinets, both of which remained fully operational throughout the testing. For routine operation, two personnel may be present, one at each workstation, and the room has a maximum occupancy limit of three people. The room volume is approximately 53m³ and has in excess of 40 air changes per hour. The cleanroom has been operational for a number of years and has demonstrated a high level of operational environmental cleanliness that easily meets the microbial and particle requirements defined for an EU grade B location³. The cleanroom is shown in **Figure 1**. It contains a fixed, wall-mounted, point-of-use particle counter, connected to a central vacuum system that continuously samples the cleanroom air at a rate of 28.3L/min (1ft³/min). Following each 1-minute sample, and hence a sample volume of 28.3L (1ft³), the number of particles at threshold size channel $\geq 0.5\mu\text{m}$ and $\geq 5\mu\text{m}$ are reported to a computer in a central control room.

Test equipment

IMD system

The BioVigilant Instantaneous Microbial Detection System (IMD-A-350) was positioned in the cleanroom. This system samples air at a rate of 28.3 L/min (1ft³/min). The air is drawn into the unit and then enters a 'concentrator', which uses the principle of a 'virtual impactor' described by Hinds⁴. Most of the particles are concentrated into 1.15L/min of air and pass down through the centre area and into the interrogation zone of the IMD-A-350 system. The remainder of the air is exhausted perpendicularly from the side of the concentrator, at a rate of 27.2L/min, and passes through a HEPA filter and vented from the system. The particles that enter into the interrogation zone are counted and recorded in the exact same manner as a standard particle counter. To permit direct comparison with the number of particles counted by a standard particle counter, these are referred to as 'total' particles in this article. This also distinguishes them from the 'biological' particles, although a number of the total



Figure 1. Non-unidirectional airflow cleanroom.

particles may also be of 'biological' origin. Simultaneously, the IMD-A-350 unit looks for fluorescence, induced by a visible laser light at a wavelength of 405nm, from any of the sized total particles. If any fluorescence above a pre-determined intensity is detected, then that particle is also recorded as a 'biological' particle. In this way, all particles, and those particles that are also considered to be of 'biological' origin, are counted and sized. When compared directly to three common types of traditional airborne microbiological sampling methods, the IMD-A-350 system was reported to have a superior detection over a range of micro-organisms nebulised into an aerosol test chamber⁵. The IMD-A-350 counter assigns both the detected total and 'biological' particles to the following channel sizes:

$\geq 0.5\mu\text{m}$ to $<1.0\mu\text{m}$
 $\geq 1.0\mu\text{m}$ to $<3.0\mu\text{m}$
 $\geq 3.0\mu\text{m}$ to $<5.0\mu\text{m}$
 $\geq 5.0\mu\text{m}$ to $<7.0\mu\text{m}$
 $\geq 7.0\mu\text{m}$ to $<10.0\mu\text{m}$
 $\geq 10.0\mu\text{m}$

To obtain a size distribution of the airborne 'biological' particles, the mid-point of each channel size was plotted against the frequency of occurrence of that size of particle. The median particle size of the distribution was obtained by cumulating the counts and then calculating the cumulated counts at each size as a percentage of the total count. The cumulative percentages were then plotted against the mid-point of the channel size to obtain the 50% cumulative count; this is the median size.

Andersen air samplers

Two identical Andersen air samplers were used to sample MCPs in the experimental cleanroom and are shown in **Figure 2**. Further details regarding this type of sampler are described by Andersen⁶. These units are cascade microbial air samplers that have six stages, each stage having 400 holes through which the sampled air passes, these holes decreasing in diameter down through the stages. Each



Figure 2. Six-stage Andersen air samplers.

stage has an agar plate below it and the impaction velocity onto each agar surface 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 median diameter and size distribution of the MCPs to be calculated by the method given by Kethley *et al.*⁷. The size distribution is usually log-normal and hence a logarithm of the D50%, of the stage above, was plotted against the percentage cumulated counts calculated on a 'less than stated size' for each stage. A regression equation was obtained of the plot, and the equivalent particle diameter at the 50% cumulative count point determined. The values for the 50% cumulative particle size impacted on each stage of the Andersen samplers, in terms of equivalent particle diameter, were obtained from published results⁴, and are as follows:

Stage 1, no stage above
 Stage 2, 9.8µm
 Stage 3, 6.2µm
 Stage 4, 3.8µm
 Stage 5, 2.2µm
 Stage 6, 0.9µm

When tested in comparison to other air samplers, the Andersen sampler is found to be one of the most efficient sampler's available⁸, especially when the intake cone is removed⁹. Consequently, both samplers were utilised without the intake cones. One sampler was used with trypticase soy agar (TSA) medium plates and incubated aerobically and the other sampler was used with Columbia Horse Blood Agar plates and incubated anaerobically. Following testing, all plates were incubated at 32.5°C for 5 days under aerobic and anaerobic conditions, respectively. The Andersen units sampled at a rate of 28.3L/min (1ft³/min), and were connected in parallel to a vacuum pump, which was located in one of the EU GGMP Grade A³ cabinets to contain the emitted particles and ensure they were not released into the cleanroom. They were placed adjacent to the IMD-A-350 unit. After sampling, the six media plates were incubated and, after incubation, the number of colonies counted and recorded.

AirTrace® microbial air sampler

To provide additional information regarding the airborne microbial concentrations, an AirTrace® slit-to-agar sampler that is routinely used to monitor the cleanroom air during manufacturing at a rate of 28.3L/min (1ft³/min) was used. It was placed in close proximity to the IMD-A-350 unit and the Andersen samplers. The evaluation of the performance of this instrument was completed by a specialist testing company, which reported it to have high collection efficiency¹⁰ when tested in the manner suggested by ISO 14698¹¹. Whyte *et al.*¹² have reported that the D50% value for this sampler using a simple analytical approach was 0.25µm, and using a computational fluid dynamics approach was 0.23µm. Standard 140mm diameter plates filled with TSA were used which, following testing, were incubated under aerobic conditions at 32.5°C for 5 days.

Lasair II® particle counter

In addition to the total particle counts measured by the IMD-A-350 system and the cleanroom point-of-use particle counter, a Lasair II® particle counter was placed in the cleanroom. This unit continuously samples air at a rate of 28.3 L/min (1ft³/min) and, after each 1-minute sample, the concentration of total particles per 28.3L (1ft³) at threshold size channels of ≥0.5µm and ≥5.0µm are reported.

Investigation undertaken

Tests with full cleanroom attire

For the first three tests, seven people in standard cleanroom attire were utilised. The cleanroom garment fabric is polyester with a fabric pore diameter of 13µm. A one-piece coverall with hood and overboots were complemented by goggles, facemask and double latex rubber gloves to provide full skin coverage. With the exception of the goggles, which are disinfected, all items are sterilised by Gamma radiation. This attire provides a very high level of personnel particle containment, and operational airborne microbial monitoring of the test cleanroom, with two personnel present and wearing this attire, gave an average count of 0.1cfu/m³.

Hence, seven people (the maximum number that could safely occupy the cleanroom) were utilised to obtain a higher concentration of airborne contamination that was within the accurate range of measurement of the microbial air samplers. The seven people maximised their contamination dispersal rates by marching on the spot, whilst swinging both arms, at a rate of approximately one beat per second for 4 minutes until the airborne particle contamination concentrations, reported by the Lasair II® counter, indicated that a 'steady state' condition had been attained. The people continued to march on the spot for a further 15 minutes as the IMD-A-350 unit, the AirTrace® sampler and Andersen samplers were all activated, and the Lasair II® counter and the fixed cleanroom particle counter continued to sample. After 15 minutes, the IMD-A-350 unit, the AirTrace® sampler and the Andersen samplers were turned off, each having sampled 424.5L



Figure 3. Testing in full cleanroom attire.

(15ft³) of air, and the microbiological media plates were removed and incubated. This process was repeated a further two times (three tests in total; Tests 1, 2 and 3). The full cleanroom testing attire is illustrated in **Figure 3**.



Figure 4. Testing in cleanroom undergarments.

Tests with cleanroom undergarments only

The testing was repeated using the same seven personnel located in the same positions within the cleanroom and moving at the same rate, but wearing only polyester/cotton cleanroom undergarments. The undergarments are laundered at a specialist garment company and a fresh set is used everyday and worn under the full cleanroom attire. Airborne particle concentrations reported by the Lasair II[®] counter indicated that 'steady state' conditions had been attained within 3 minutes. The people continued to march on the spot for a further 5 minutes as the IMD-A-350 unit, the AirTrace[®] sampler and the Andersen samplers were all activated, and the Lasair II[®] counter and the fixed cleanroom particle counter continued to sample. After 5 minutes of sampling, a volume of 141.5L (5ft³) had been sampled, and the IMD-A-350 unit, the AirTrace[®] sampler and the Andersen samplers were turned off. The media plates were removed, incubated and the colonies counted. This process was repeated a further two times (three tests in total; Tests 4, 5 and 6). The cleanroom undergarments testing attire is illustrated in **Figure 4**.

Results

For both test conditions (using full cleanroom attire or cleanroom undergarments), the three sets of results were found to be in good agreement with each other. For simplification and clarity, the averages for both test conditions have been reported throughout and all data is given as counts per m³.

Total particle counts

The average counts of $\geq 0.5\mu\text{m}$ and $\geq 5.0\mu\text{m}$ diameter total particles that were simultaneously recorded by the IMD-A-350 system and the Lasair II[®] particle counter are shown in **Table 1**. The total particles counted by the IMD-A-350 system is also given as a percentage of those counted by the Lasair II[®] and shown in parentheses. These values show that the IMD-A-350 unit counted between 60% and 79% fewer total particles than counted by the Lasair II[®] system. The particle counts recorded by the fixed cleanroom particle counter were all within 10% of the counts recorded by the Lasair II[®] unit, and, therefore, not recorded in **Table 1**.

Also shown in **Table 1** are the reductions in particles, produced by wearing the full cleanroom attire compared to those produced when wearing only the cleanroom undergarments. It can be seen that although the IMD-A-350 instrument counted less total particles than the Lasair II[®], the reductions in particle counts when wearing the complete cleanroom attire compared to the undergarments, are reasonably similar for both instruments.

'Biological' and microbiological counts

The average number of MCPs recovered by the Andersen and AirTrace[®] samplers, compared to the average number of 'biological' particles recorded by the IMD-A-350 unit, are shown in **Table 2**. For the tests performed with personnel wearing full cleanroom attire, the average

Table 1. Averaged total particle counts per m³. Shown in parentheses are the total particles counted by the IMD-A-350 system as a percentage of the total particles counted by the Lasair II® sampler.

Garments (test numbers)	Air sampler counts per m ³			
	Lasair II®		IMD-A-350	
	≥0.5µm	≥5.0µm	≥0.5µm	≥5.0µm
Full cleanroom attire (Tests 1, 2 and 3)	15,991	1563	4872 (30.5%)	330 (21.1%)
Cleanroom undergarments (Tests 4, 5 and 6)	158,377	22,846	62,706 (39.6%)	7102 (31.0%)
Reduction in inert particles; cleanroom attire vs. undergarments	9.9	14.6	12.9	21.5

number of aerobically-incubated MCPs recovered by the AirTrace® air sampler was in good agreement with the number of aerobic MCPs recovered by the Andersen samplers and provides confidence regarding the performance of both of these instruments.

It is normal practice in cleanrooms to incubate samples aerobically. However, there are a number of microbes that only grow anaerobically and may be part of the explanation as to why the count of ‘biological’ particles was so much higher than MCPs. Therefore, as described previously, half of the samples from the Andersen samplers were incubated anaerobically. However, there is a group of micro-organisms that will grow in both anaerobic and aerobic conditions, which are known as ‘facultative’ microbes. Although it is not scientifically correct to add both types of micro-organisms to obtain a combined total number of MCPs, doing so provides a ‘best case’ comparison with the ‘biologicals’ counted by the IMD-A-350 system. For this reason, and for further simplification, this is the approach that has been used.

It can be seen in **Table 2** that the counts of ‘biological’ particles were 94 times greater (2725 vs. 29) than the total counts of the MCPs when full cleanroom attire was worn, and 7 times greater (25,574 vs. 3841) when cleanroom undergarments were worn. Also shown in **Table 2** are the reductions in airborne ‘biological’ particles and MCPs when full cleanroom attire was worn in comparison to cleanroom undergarments. The reduction of ‘biological’ particles measured by the IMD-A-350 was 9 times

(25,574 vs. 2725) and the reduction of MCPs measured by the Andersen samplers was 132 times (3841 vs. 29).

‘Biological’ particles and MCPs as a proportion of the inert particle counts

The airborne counts obtained in the cleanroom from the IMD-A-350 unit and the Andersen and Lasair II® samplers are given in **Table 3**. Also given in parentheses are the number of biological particles as a percentage of the ≥0.5µm and ≥5.0µm total particles counted by the same IMD-A-350 instrument, as well as the number of MCPs recovered by the Andersen samplers as a percentage of the ≥0.5µm and ≥5.0µm particles counted by the Lasair II® unit. These results show that the ‘biological’ particles are a much higher percentage of the total particles than the corresponding MCPs. Additionally, it can be seen that the concentration of ‘biological’ particles is more than the concentration of associated ≥5.0µm particles, whereas the concentration of MCPs remains less than the number of ≥5.0µm particles.

Size distributions of ‘biological’ particles and MCPs

The size distributions of ‘biological’ particles recorded by the IMD-A-350 system and the MCPs recovered by the Andersen samplers are shown in **Figure 5**. For the Andersen samplers, these data are only for the undergarment tests as there were insufficient MCPs present to record accurate results when using the full

Table 2. Averaged airborne ‘biological’ and microbiological counts per m³ when full cleanroom attire and only cleanroom undergarments were worn.

Garments (test numbers)	Air sampler counts per m ³				
	IMD-A-350	Andersen			AirTrace®
	‘Biologicals’	Aerobic incubation	Anaerobic incubation	Total MCPs	Aerobic incubation
Full cleanroom attire (Tests 1, 2 and 3)	2725	26	3 ^a	29	24
Cleanroom undergarments (Tests 4, 5 and 6)	25,574	2930	911	3841	TNTC ^b
Reduction in ‘biological’ particles or MCPs with full cleanroom attire compared to undergarments	9	–	–	132	–
‘Biologicals’:MCPs ratio	Full cleanroom attire 2725: 29 = 94:1 Cleanroom undergarments 25,574: 3841 = 7:1				

^aOnly 1cfu was recovered with the actual sample volume of 424.5L

^bColonies were above 250 per plate and too numerous to count accurately

cleanroom attire. The 'biological' particle data, for both the full cleanroom attire and cleanroom undergarments, are also shown in **Figure 5**, and show identical profiles.

The calculated median particle diameter values are shown in **Table 4**. Also shown in the last column of this table are the actual numbers of 'biological' particles and MCPs recorded, the low numbers being the reason why the size distributions of MCPs associated with the full cleanroom attire experiments have not been included in **Figure 5**, or the medians in **Table 4**.

Sources of 'biological' particles

Tests were carried out on a variety of materials found in the cleanroom to determine the number of 'biological' and total particles that were dispersed. The materials tested were as follows: sterile and unsterilised hood used in the full cleanroom attire, fresh undergarment top, sterile cleanroom wipe, sterile 70% isopropyl alcohol in water for injection (70% IPA), sterile latex gloves and ungloved hands. The testing was performed in a horizontal unidirectional airflow cabinet.

Table 3. Biological' particles/m³ and MCPs/m³ in cleanroom air. Shown in parentheses are both the 'biological' and MCP counts as a percentage of total particle counts, at diameters $\geq 0.5\mu\text{m}$ and $\geq 5.0\mu\text{m}$.

Garments (test numbers)	Air sampler counts per m ³					
	IMD-A-350			Andersen	Lasair II®	
	'Biologicals'	$\geq 0.5\mu\text{m}$	$\geq 5.0\mu\text{m}$	Total MCPs	$\geq 0.5\mu\text{m}$	$\geq 5.0\mu\text{m}$
Full cleanroom attire (Tests 1, 2 and 3)	2725	4872 (55.9%)	330 (826%)	29	15,991 (0.2%)	1563 (1.9%)
Cleanroom undergarments (Tests 4, 5 and 6)	25,574	62,714 (40.7%)	7102 (360%)	3841	158,377 (2.4%)	22,846 (16.8%)

Table 4. Median 'biological' particles and MCPs sizes.

Garments (test numbers)	Air sampler	Sample type	Median particle size (μm)	Actual number of 'biologicals' or MCPs
Full cleanroom attire (Tests 1, 2 and 3)	IMD-A-350	'Biological'	1.2	1158
	Andersen	Aerobic	Not determined	11
		Anaerobic	Not determined	1
Cleanroom undergarments (Tests 4, 5 and 6)	IMD-A-350	'Biological'	1.2	3622
	Andersen	Aerobic	4.6	415
		Anaerobic	7.7	129

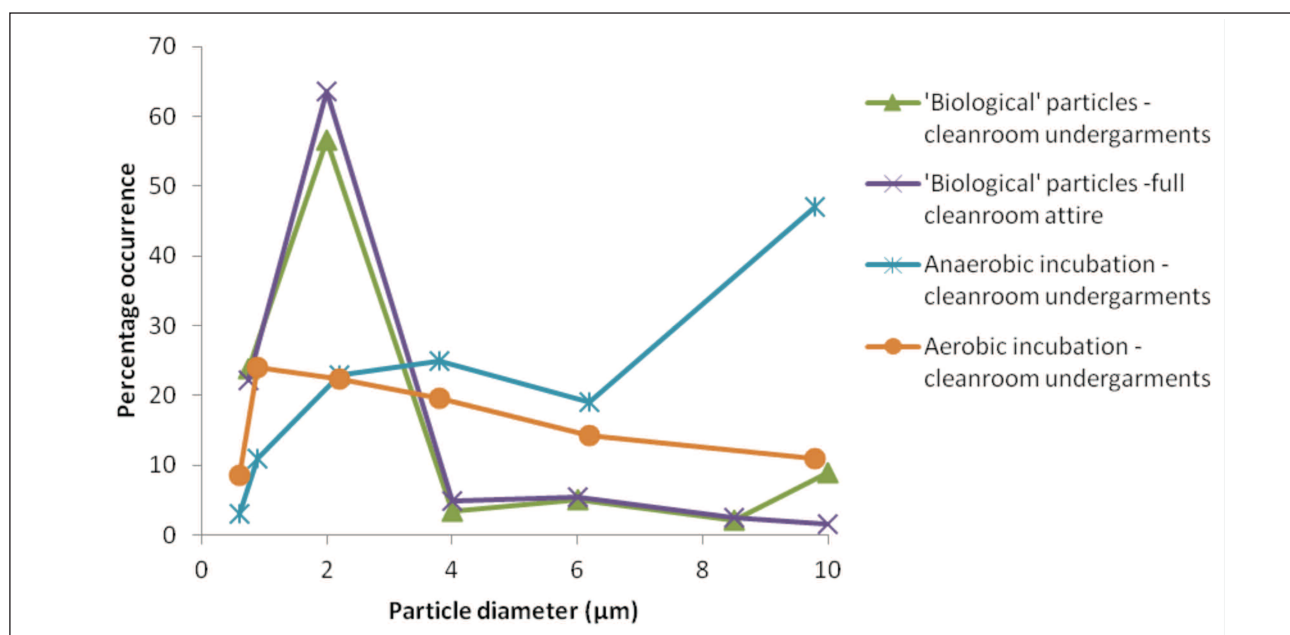


Figure 5. Size distributions of 'biological' particles (IMD-A-350 unit) and MCPs (Andersen samplers).

The materials to be tested were transferred into the cabinet using, where applicable, sealed standard wrappings provided by the suppliers. Depending on the material sampled, variations were made to the method of counting the dispersion of particles. In the case of the articles of clothing and the wipe, the material was unwrapped using an appropriate technique to prevent contamination, and touched gently over the air intake of a remote flexible pipe connected to the IMD-A-350 unit. The resultant counts were recorded as a single event. An additional method was used to test the sterile cleanroom hood where the hood was agitated above the intake pipe. The sterile 70% IPA was sprayed above the intake to the pipe. Both the gloved and ungloved hands were tested by rubbing them together above the intake pipe. These

procedures were performed three times for each material tested and the averaged results are summarised in **Table 5**. It can be seen that all of these materials dispersed 'biological' particles, and when expressed as a percentage of the total number of $\geq 0.5\mu\text{m}$ and $\geq 5.0\mu\text{m}$ particles measured by the IMD-A-350, they represent a significant proportion (between 14% and 74% in the case of $\geq 0.5\mu\text{m}$ particles), similar to that recorded in the cleanroom by the IMD-A-350 system (see **Table 3**).

Discussion

A comparison was made in an EU GMP Grade B³ pharmaceutical cleanroom of the airborne concentration of MCPs, 'biological' particles and total particles. Shown

Table 5. Total and 'biological' particle counts from typical cleanroom materials recorded by the IMD-A-350 unit. Shown in parentheses are the 'biological' particle counts as a percentage of IMD-A-350 total particle counts, at diameters $\geq 0.5\mu\text{m}$ and $\geq 5.0\mu\text{m}$.

Material	Material status	Testing type	IMD-A-350 Sampler		
			Number of particles		
			$\geq 0.5\mu\text{m}$	$\geq 5.0\mu\text{m}$	'Biologicals'
Sterile cleanroom hood (polyester)	Laundered by a specialist cleanroom garment management company, under cleanroom conditions using purified water (<5cfu/ml). Garment has a total pre-irradiation bioburden of <29cfu per hood (0.05cfu/cm ²). Sterilised by irradiation to provide a sterility assurance level of 10 ⁻⁶	Direct contact with sampler air intake pipe	330 (60%)	29 (682%)	198 –
		Agitated above to release airborne particles into the intake pipe	21 (43%)	3 (300%)	9 –
Unsterile cleanroom hood (polyester)	Laundered by a specialist cleanroom garment management company, under cleanroom conditions using purified water (<5cfu/ml). Garment has a total bioburden of <29cfu per hood (0.05cfu/cm ²)	Direct contact with sampler air intake pipe	67 (73%)	6 (816%)	49 –
Cleanroom undergarments (cotton/ polyester)	Laundered by a specialist cleanroom garment management company, under cleanroom conditions using purified water (<10cfu/ml). Garment has a total bioburden of <5cfu per 55mm contact plate (0.2cfu/cm ²)	Direct contact with sampler air intake	602 (54%)	97 (336%)	326 –
Sterile cleanroom wipe (polyester)	Prepared by a specialist cleanroom garment management company under EU grade A cleanroom conditions using purified water. Wipe has a total pre-irradiation bioburden of <1cfu per wipe (0.09m ²) and an endotoxin limit of <1EU. Sterilised by irradiation to provide a sterility assurance level of 10 ⁻⁶	Direct contact with sampler air intake pipe	961 (14%)	11 (12%)	131 –
Sterile 70% IPA (aerosol delivery)	Produced using water for injection (WFI) and the solution and propellant filtered via a sterilising grade filter (0.2 μm) under cleanroom conditions by a specialist company. Solution has a total pre-irradiation bioburden determined to be <0.4cfu per 360ml. Sterilised by irradiation to provide a sterility assurance level of 10 ⁻⁶	Liquid sprayed into air near the intake pipe	3182 (74%)	2 (1174%)	2348 –
Sterile glove (latex, powder free)	A pre-irradiation bioburden of <5cfu per glove. Sterilised by irradiation to provide a sterility assurance level of 10 ⁻⁶	Hands rubbed above the intake pipe	61 (28%)	9 (188%)	17 –
Ungloved hands	Hands washed approximately 3 hours before the test with standard non-biocidal cleaning agent. Typically, unwashed hands will have a high bioburden	Hands rubbed above the intake pipe	26 (31%)	4 (200%)	8 –

in **Table 1** is a comparison of the airborne concentrations of total particles measured by the IMD-A-350 unit and Lasair II® particle counter, when wearing either full cleanroom attire or cleanroom undergarments. The fixed cleanroom point-of-use particle counter results were not included as they were almost identical to those recorded by the Lasair II® counter. It was found that the IMD-A-350 unit recorded between 60% and 79% fewer particles than counted by the Lasair II®. This difference can be explained by the method used by the IMD-A-350 to concentrate particles into a smaller air volume using 'virtual' impaction. This method will lead to particle losses, as some smaller particles will fail to be collected and some larger particles will be impacted onto the internal surfaces of the unit. However, although the counts of total particles were different between the IMD-A-350 and the Lasair II®, both instruments gave similar reductions of total particles when full cleanroom attire was worn in place of cleanroom undergarments.

The results in **Table 2** show that when full cleanroom attire was worn, the IMD-A-350 instrument counted 94 times more 'biological' particles than MCPs sampled by the Andersen air samplers. This was confirmed by the MCP counts measured by the AirTrace® sampler. However, when personnel wore cleanroom undergarments, the difference was only 7 fold. The only change between these experiments was the clothing worn, and hence the reason for this difference must presumably be greater quantities of fluorescent particles dispersed by the full cleanroom attire counted as 'biological' particles by the IMD-A-350 instrument. It should be noted that if the reduction of the number of total particles recorded by the IMD-A-350 unit compared to the Lasair II® is taken into consideration, then larger proportional differences between the number of 'biological' particles and MCPs would be expected.

Further analysis of the results given in **Table 2** show that the total number of MCPs recovered from the cleanroom air when personnel wore full cleanroom attire was 132 times less than when cleanroom undergarments were worn. Unpublished work carried out at AstraZeneca Macclesfield using a personnel dispersal chamber of the type described by Whyte *et al.*¹³ gave very similar differences. It might, therefore, be expected that the IMD-A-350 unit would give a similar reduction of 'biological' particles but only a 9-fold decrease was measured. This result is also contrary to what might be expected from the results shown in **Table 1**, which give relatively uniform reductions of total particles measured by the IMD-A-350 unit and Lasair II®, when full cleanroom attire was worn instead of cleanroom undergarments. Again, the only reason for the difference was the clothing worn, and this anomalous result is, again, considered to be caused by a difference in the rate of dispersion of fluorescent 'biological' particles from the two types of cleanroom clothing.

Given in **Table 3** are the 'biological' particles as a percentage of the associated total particle counts measured by the IMD instrument, as well as the MCPs as a percentage of the total particles measured by the Lasair

II® instrument. The 'biological' particles were found to account for over 40% of the $\geq 0.5\mu\text{m}$ particles present in the cleanroom air. It appears unlikely that such a high percentage of particles in a cleanroom would be of a microbial nature, and the percentage of MCPs normally found during routine sampling in cleanrooms are closer to that found in these experiments, i.e. 0.2% and 2.4% of the $\geq 0.5\mu\text{m}$ particles. **Table 3** also shows another unexpected result in that there were up to 8.26 times more 'biological' particles than particles $\geq 5.0\mu\text{m}$ in the cleanroom air.

The size distributions of 'biological' and MCPs are shown in **Figure 5**. Microbes in the air of occupied rooms are derived from personnel who disperse skin cells that may carry micro-organisms^{14,15} and several hundred MCPs per minute pass through cleanroom clothing¹² and into the cleanroom air. Micro-organisms are usually found in cleanrooms attached to skin particles (and to a much lesser extent to clothing fibres) and are best known as MCPs. Most of the MCPs found in skin fragment experiments were reported by Lundholm¹⁶ to be greater than $5\mu\text{m}$. Noble *et al.*¹⁷ reported the average diameter of MCPs to vary between about $8\mu\text{m}$ and $16\mu\text{m}$, and Whyte¹³ suggested that a reasonable size to assume for the average diameter of MCPs was $12\mu\text{m}$.

It can be seen in **Figure 5** that the sizes of MCPs recorded by the Andersen sampler were much larger and more evenly distributed than the 'biological' particles. The 'biological' particles had a definite peak and a median diameter of $1.2\mu\text{m}$. This diameter is very much smaller than the MCPs found in the experimental cleanroom and reported in other occupied rooms^{13,16,17}. Information obtained from the manufacturers about the 'concentrator' in the IMD-A-350 unit shows that it has a 50% recovery at a particle cut-off size of $1.0\mu\text{m}$ and, below that size, the recovery efficiency drops below 50%. This is a likely explanation for the size distribution of the 'biological' particles peaking at $1.2\mu\text{m}$. The median sizes of aerobically- and anaerobically-incubated MCPs measured in these experiments were $4.6\mu\text{m}$ and $7.7\mu\text{m}$ respectively, which, although at the lower end of sizes, are within the expected range. The different sizes of 'biological' particles and MCPs raise doubt as to whether the IMD-A-350 instrument was mainly counting MCPs.

It is known that some types of microbial air samplers have a low collection efficiency⁸, and that sampling in cleanrooms does not normally include the measurement of microbes that grow anaerobically. In this investigation microbial samplers were used that had high collection efficiencies, and the total counts included microbes that were incubated anaerobically. However, these additional measures failed to account for the much larger counts of 'biological' particles found by the IMD-A-350 unit. The IMD-A-350 unit would be expected to count a larger number of micro-organisms compared to the traditional method, as 'biological' particles are likely to include non-viable cells, microbes that do not grow using normal growth methods and, perhaps, fragments of cells. However, this additional detection ability would be highly unlikely to fully account for the very much higher number of 'biological' particles compared to MCPs.

Numerous common materials, such as white paper, inks, dyes and certain polymers are commonly known to fluoresce. A wide variety of sterile cleanroom materials, such as clothing, skin, IPA spray, etc., were, therefore, tested to find out if they will disperse fluorescent particles that are identified by the IMD-A-350 sampler as 'biological' particles. Although these materials were sterile they would contain microbes prior to sterilisation, and the dead microbes may be counted as 'biological' particles. The materials were, therefore, prepared in highly-controlled clean environments to ensure that there was a very low bioburden prior to sterilisation.

Table 5 confirms that most of the sterile materials used in cleanrooms disperse particles that fluoresce and are counted as 'biological' particles. The percentage of 'biological' particles ranged from 14% to 74% of the $\geq 0.5\mu\text{m}$ particles, which is similar to the percentages of 'biological' particles recorded in the cleanroom by the IMD-A-350 sampler. It was concluded that a considerable number of particles recorded by the IMD-A-350 unit as 'biological' particles were particles of non-microbiological origin, and many times more numerous than actual MCPs.

Conclusions

The pharmaceutical industry is, quite rightly, most concerned about airborne particles that may deposit onto, or into, products and then proliferate to cause contamination, spoilage or infection. Particles that carry viable micro-organisms are the main concern, and other types of particles, such as dead or fragmented microbes, or inanimate particles that fluoresce when exposed to laser light, are of much less concern.

It is accepted that the well-established monitoring methods used at present in cleanrooms can only determine a proportion of the viable micro-organisms present in cleanroom air. However, these methods work reasonably well in isolating the micro-organisms most commonly found in cleanrooms, i.e. the skin flora, as well as indicating changes in the concentrations of environmental micro-organisms. Nonetheless, due to the relatively low concentrations of airborne MCPs, and the delay in obtaining results caused by the slow formation of colonies of microbes, a method that determines airborne counts of MCPs in real time would be a considerable asset to the monitoring of cleanrooms.

For the type of cleanroom investigated, the count of 'biological' particles appears to be unrealistically high in comparison to both the MCPs and the total particles. The 'biological' particles also had a different size distribution and were smaller than the MCPs. The explanation for these observations appears to be that large quantities of particles emitted from a variety of the materials commonly found in this type of cleanroom, such as garments, skin and IPA spray, are indistinguishable from 'biological' particles.

Some acceptance of 'biological' particles that were of a non-microbiological origin would be reasonable, as long as significant changes in the concentration of MCPs could be detected. However, the experiments discussed in this

article showed that the variation in the numbers of MCPs produced by a change in clothing was not accompanied by an analogous change in the concentration of 'biological' particles, and would go undetected, being effectively masked by non-microbiological fluorescent particles. Accordingly, monitoring of this type of cleanroom to confirm appropriate microbiological contamination control with such an instantaneous microbiological monitoring system is not considered to be adequate. However, if the IMD system could be improved to accurately differentiate between micro-organisms and non-viable fluorescent particles, and the dispersion of fluorescent particles from non-microbiological sources reduced, then this system could provide a desirable cleanroom airborne monitoring method. This may be the case in EU GMP Grade A³ critical zones, as there would be an absence of personnel and sources of non-viable fluorescent particles. Further investigations in such areas would be useful.

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