Particulate Science and Technology
An International Journal

ISSN: 0272-6351 (Print) 1548-0046 (Online) Journal homepage: http://www.tandfonline.com/loi/upst20

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To cite this article: I. Tovena Pecault, M. Thibaudon, J. Clertant & P. Godefroy (2018) Macroparticles monitoring for biocontamination prevention, Particulate Science and Technology, 36:7, 908-912, DOI: 10.1080/02726351.2017.1346021

To link to this article: https://doi.org/10.1080/02726351.2017.1346021

Accepted author version posted online: 12 Jul 2017.
Published online: 27 Nov 2017.

PARTICULATE SCIENCE AND TECHNOLOGY
2018, VOL. 36, NO. 7, 908–912
https://doi.org/10.1080/02726351.2017.1346021

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Macroparticles monitoring for biocontamination prevention

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ABSTRACT

This study investigated a potential correlation between deposited macroparticles >5µm and bioaerosols. Bioaerosols were detected using both passive (culture plates) and dynamic sampling (biocollectors). Bioaerosols were then revealed visually on culture plates after incubation and counted in colony forming unit (CFU)/cm^2. Macroparticles were counted on line by CLEAPART-100 (patent CEA – Aix Marseille University – Winlight System). It enables continuous monitoring of the deposit of macroparticles on surfaces. We have shown that those macroparticles could be strongly correlated to viable particle measurements (bacteria and mould) after dynamic sampling by biocollectors. As viable particle measurements require an incubation period of at least 48 hours, it is easy to understand the advantage in continuous macroparticle done by CLEAPART-100. CLEAPART-100 is therefore an instrument which enables the alert to be raised in relation to any contamination or biocontamination events.

KEYWORDS

Bioaerosol; biocontamination; deposition; macroparticle; monitoring

1. Introduction

Bioaerosols, including bacteria (*Micrococcus, Staphylococcus, Bacillus, Pseudomonas,* etc.) and mold, present special health hazards due to the risk of infection. A certain number of studies were performed to correlate the concentrations in bioaerosols and in particles, whether in a clean controlled environment (Oxborrow et al. 1975; Ljungqvist and Reinmüller 2006), or indoor (Tham and Zuraimi 2005; Napoli 2012a,b) since the inert and viable particles are considered as the main causes of asthma, respiratory diseases, reduced productivity, and sick building syndrome (Husman 1996). Viable particles are particles that consist of, or support, one or more live microorganisms and all viable particles are not cultivable. In these studies, the aerosols were generally measured with dynamic sampling systems with continuous light scattering particle counting using optical counters. They are not focused on the deposited particles. However, for the bioaerosol measurements, two types of samplings are performed: passive for the "deposited" bioaerosols and dynamic for the "impacted" bioaerosols. Several studies have been performed to compare these two methods and the results are discordant. In certain studies, the two dynamic and passive bioaerosol sampling methods give results which may be correlated (Orpianesi et al. 1983; Perdelli et al. 2000) which is not the case in other studies (Sayer, MacKnight, and Wilson 1972; Petti, Iannazzo, and Tarsitani 2003). Nevertheless, the bioaerosol sampling method should be chosen according to its representative nature in relation to the risk of biocontamination: airborne (dynamic sampling in biological particle/m^3) or sedimented (in biological particle/m^2) as recommended by C. Napoli in his article (Napoli 2012a,b). In cleanroom, macroparticles are defined as particles larger than 5 µm (ISO 14644-1/Annex C). Unlike in previous studies focused on the airborne particles, deposited macroparticles >5 µm are measured on line, with the CLEAPART-100 in this study. For the last 10 years or so, new commercial devices allow continuous monitoring of the deposit of these macroparticles on surfaces: APMON, PDM, or CLEAPART-100 (Tovena Pecault et al. 2016). CLEAPART-100 is the only system which enables imaging and counting of deposited particles from 5 µm without an upper size limit. However, these deposited macroparticles are precisely those which represent a major risk in health-related industries and hospitals. In 1955, Wells discovered that airborne germs were actually transported by particles (Wells 1955). In 1996, Whyte recommended that the pharmaceutical community, in particular, monitor deposited particles rather than aerosols (Whyte 1996). While most of those particles are composed of inert materials, some are of biological origin (Owen, Ensor, and Sparks 1990). Airborne microorganisms or bioaerosols consist of all airborne particles of biological origin, i.e., bacteria, fungi, viruses, and pollen and their fragments (Cox and Wathes 1995). For the bioaerosols, and given the lack of consensus at international level on the dynamic or passive sampling method for biocontaminants, both methods are used in this study: dynamic sampling thanks to three types of biocollectors (Mas 100, Sampl'Air, Air Ideal) and static sampling with culture plates. Whether the static or dynamic sampling mode is used, in both cases the bioaerosols are collected in culture plates with a culture environment which favors their growth when they are incubated under specified conditions. Another parameter which is important in such comparison is the culture media which might be different from one study to another. Moreover, it means that only the cultivable viable bioaerosol is compared to the deposited macroparticles. The aim of this study is to assess whether we can correlate the deposited

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macroparticles to cultivable bacteria and molds to prevent environmental biocontamination event.

2. Methods

2.1. Experiments

The experiment was performed in June in a training room (relative humidity of 50% maintained at temp 21–23°C) (Figure 1) and not in a clean room; the objective was to measure the deposited particles and biocontaminants appropriately. There is no special reason for performing the experiment in June even if we know that there are relatively more molds in June. All the equipment was positioned on a large table at a height of 1 m in the center of which was the CLEAPART-100. Four culture plates integrating a nonselective tryptic soy agar (TSA) were positioned 50 cm from the CLEAPART-100 and the four biocollectors (Mas 100, Sampl'Air, and 2 Air Ideal diametrically opposed) were positioned at around 1 m away. The measurements began at 9.33 am and ended at 11.18 am same day, i.e., nearly 2 h of sampling for all the resources.

2.2. Passive biocontamination sampling

Four TSA culture plates 90 mm in diameter were used to collect the biocontaminants before incubation.

2.3. Active biocontamination sampling

All the three biocollectors used are based on principle of impacting the bioaerosols through a specific grill then collection on a 90 mm culture plates with a TSA-type nonselective culture medium:
–Mas 100,
–Sampl'Air,
–Air Ideal (2 specimen).

Tryptic soy agar is a nonselective medium as it provides enough nutrients to support the growth of a wide range of microorganisms (yeasts, molds, aerobic, anaerobic, etc.). The sampling rate is 100 l/min for each of them to facilitate comparison.

Between 9:33 am and 11:18 am, 16 culture plates were collected from each biocollector to be incubated to count the number of bacteria and molds (total of 64 culture plates).

2.4. Viable particles counting

After collection, the culture plates (active and passive sampling) are then incubated at 32.5°C for 48 h to count the bacteria and mold. Culture results are reported as the number of colony forming units (CFU) for each bacteria and mold category and then adjusted by plate surface to obtain deposited bioaerosol concentration (CFU/cm^2). Blank samples have been used but not analyzed because below the detection limit of 1 CFU per culture plate. Few samples (less than 8%) were lost because overgrown.

2.5. Particle deposit

CLEAPART-100 is an imaging device and continuous counter for particles larger than 5 µm. It allows users to detect particles and sort them by size, starting from 5 µm. The detector comes in cube form. It has a high flatness glass surface (100 cm^2 surface) which collects particles, which are then detected by a mobile optical camera positioned underneath the glass plate, on X and Y axes (Figure 2). The particles are then classified continuously according to a preestablished classification (>5, >15, >25, >50, >100 µm).

CLEAPART-100 was developed in the context of cleanliness monitoring for Mégajoule Laser by the CEA-CESTA in collaboration with the University of Aix Marseille and Winlight System [patent (Tovena Pecault et al. 2015)]. The counting process lasts for 5 min. The uncertainty of the CLEAPART-100 counting has been assessed using a test pattern on the CLEAPART-100 window. The pattern represents a network of thousands of particles 10 µm in size. We have then

Figure 1. Layout of the different items of measurement equipment: in the center the CLEAPART-100, four culture plates 50 cm away, then four biocollectors 1 m away.

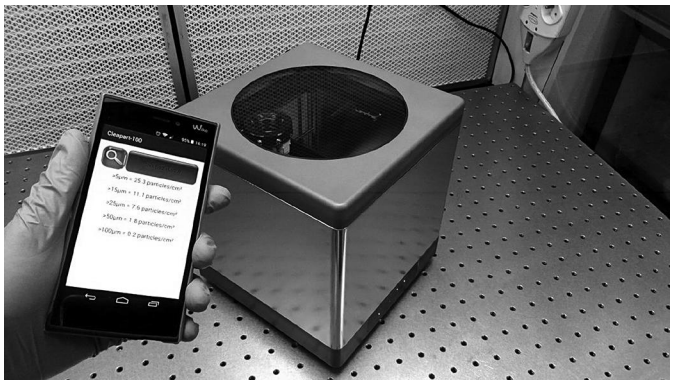

Figure 2. CLEAPART-100 v1 for continuous display of the counting and classification results for particles >5, >15, >25, >50, >100 µm on a smartphone (left side).

compared the number of particles counted by CLEAPART-100 to the predefined number of particles on the pattern. So doing, the CLEAPART-100 uncertainty is ±5%.

3. Results

3.1. Deposited particle contamination

CLEAPART-100 gives for each particle size, i.e., >5, >15, >25, >50, >100 µm, the number of deposited particles/cm^2, then the deposition rates are calculated and tabulated in Table 1.

The particle contamination rates depend on the particle size (7.7 part/cm^2/h for sizes between 5 and 15 µm and 0.26 part/cm^2/h for sizes >100 µm for example).

3.2. Bioaerosol collected by deposition

The deposited biocontaminants results obtained on culture plates (BP) after incubation are presented in Table 2. The related average biocontamination rates are then calculated. They are compared to CLEAPART-100 particle deposition.

The biocontamination rates (bacteria + mold) are between 0.11 and 0.21 CFU/cm^2/h after 1 h 44 sec exposure in the tested environment. The average rate is 0.15 ± 0.1 CFU/cm^2/h. We consider that the distribution of results is normal and then the expanded uncertainty is the standard deviation (σ) multiplied by a coverage factor of two. The coverage probability is then near 95.5%. However, even if the static biocontamination rates are of the order of scale of particle contamination rates for macroparticles >100 µm, this is not the case for smaller particles (Section 4).

Table 1. Total or divided sedimented particle rates in part/cm^2/h at the end of experiment at 11.18 am (in cumulative mode >x µm or differential mode $x < d < y$; x and y being the class limits).

Particle size	Rate(total part/cm^2/h)	Particle size	Rate (part/cm^2/h)
>5µm	23	$5 < d < 15$ µm	7.7
>15µm	15.3	$15 < d < 25$ µm	3.8
>25µm	11.5	$25 < d < 50$ µm	8.5
>50µm	3	$50 < d < 100$ µm	2.74
>100 µm	0.26	>100 µm	0.26

Table 2. Bioaerosol results obtained on culture plates.

	CFU for BP or particle (for CLEAPART-100)	CFU (or particle for the last row) cm^2	Rate (CFU or particle-last row cm^2/h)
BP to A2 (Air Ideal 2 biocollector)	12	0.19	0.11
BP to SA1 (Sampl'Air biocollector)	23	0.36	0.21
BP to M1 (Mas 100 biocollector)	19	0.30	0.17
BPto A1 (Air Ideal 1 biocollector)	14	0.22	0.13
Average BP	*17*	*0.27*	*0.15*
Standard discrepancy	*5*	*0.08*	*0.05*
CLEAPART-100 particles	60,500 ± 605	60.5 ± 0.6	35.00 ± 0.3

3.3. Biocontamination collected by the biocollectors

The biocollectors which are dynamic samplers at over 100 l/min should enable more measurable bioaerosols to be concentrated, the statistic to be increased and so the reliability of the comparison between bioaerosols and sedimented particles. Between 9:33 am and 11:16 am, 16 Petri dishes were collected in each biocollector to be incubated to detect the number of bacteria and molds present on each agar plate (CFU/cm^2). In the case of sampling through Air Ideal 1, the precise counting of the bacteria and mold on the agar plate could not be performed as the colonies could no longer be detected individually; they were *overgrown*. Thus only the results from the Air Ideal 2, the Mas 100, and the Sampl'Air were considered on Figure 3. Most of the biocontamination collected concerns bacteria (less than 1% mold).

The highest biocontamination rates are obtained at the start of the experiment (41 ± 3 CFU/cm^2/h), then there is a gradual fall before a slight increase from 11 am (13 ± 3 CFU/cm^2/h), confirmed at 11.10 am (18 ± 2 CFU/cm^2/h).

4. Discussion

In nonindustrial indoor environment with maintained conditioned air, bioaerosols are mainly bacteria of human origin (Stetzenbach 1997). In particular, activities like talking, sneezing, coughing, walking can generate airborne biological particulate matter. Moreover, it is well known that particles of skin with intrinsic bacteria are dispersed by the human body (Rothman 1954; Noble, Lidwell, and Kingston 1963; Clark, Cox, and Lewis 1971). That is why the high biocontamination rates in Figure 3 are associated with the presence and stirring of two experimenters in the premises at the start and end of the experiment. Outside these two periods, there was only one static experimenter in the room. These results confirm that a significant part of the microbiological aerosols are brought in by humans into a closed, clean, and air conditioned environment.

In Table 2, the total particle concentration obtained by the CLEAPART-100 at the end of test is 60.5 part/cm^2 while the average biocontamination level is 0.27 ± 0.16 CFU/cm^2 (2σ), which represents barely 0.4%. This is in keeping with other work which shows that less than 1% of aerosols are microbiological in nature (Tham and Zuraimi 2005). In the case of this study by Tham and Zuraimi, the aerosol measurements were taken between 0.3 and >7.5 µm, but the correlation study

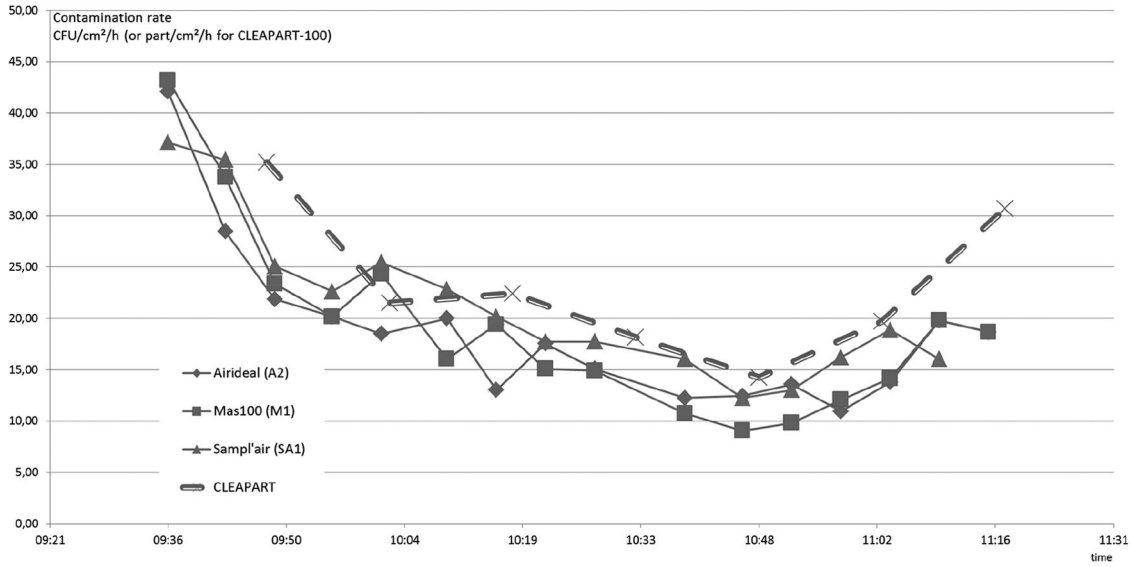

Figure 3. Comparison of the instant total particle contamination rates (size >5 µm) measured with CLEAPART-100 and the speeds in viable particles measured after dynamic sampling by the three biocollectors (Air Ideal, Mas 100, Sampl'Air).

between aerosols and biocontamination was performed from 0.65 µm. In this study, Tham and Zuraimi showed that in a closed environment, the viable particle fraction was the highest for maximum-sized particles, i.e., in their study >7.5 µm with one valid bacterium out of around 400 particles, i.e., 0.25%.

The results obtained with three different biocollectors (Table 2) show a quite low dispersal in the bioaerosol results, of maximum 25%. This is explained by the physical characteristics of similar biocollectors, i.e., in particular, the same rate of 100 l/min, which conforms to the recommendations of Napoli, Marcotrigiano, and Montagna (2012a), Napoli (2012b). In fact, he has shown that active and passive biocontamination results could be correlated to aerosol results, in the two states in activity and at rest if the same precise protocol was used. The particle contamination rates may be compared to the viable particle data after normalization on the collection surface and exposure time. The particle contamination rates (part/cm^2/h) are then compared to the biocontamination rates (CFU/cm^2/h). In particular, this enables particle contamination events to be highlighted, whether the particles are viable or not.

The particle contamination rates given by the CLEAPART-100 (viable and nonviable deposited particles >5 µm) are slightly higher (less than 10%) than the dynamic biocontamination rates given by the biocollectors (Sampl'Air, Air Ideal, Mas 100) but show the same trends (Figure 3). They confirm that the major contamination events occurred at the start and end of the experiment related to the presence of experimenters. The bioaerosol concentration depends on the number of operators, their activity, and their clothing. Most of the viable particle contamination may be associated with skin fragments. Skin flakes move freely through the clothing and are then carried up in the airstreams (Lewis et al. 1969). The skin fragments would have equivalent diameters of 4–20 µm (Noble, Lidwell, and Kingston 1963). Lundholm (1982) specifies that of the skin fragments, 48% of them which carry bacteria have a diameter over 8.2 µm. The correlation between bacteria and aerosols depends strongly on the temperature and hygrometry conditions. The higher the temperature the lower the hygrometry and the more the correlation between bioaerosol and particles will move toward large particles. In fact, at a higher temperature or lower hygrometry, the desiccation of the small particles is too fast to keep the microorganisms alive (Tham and Zuraimi 2005).

5. Conclusion

In nonindustrial indoor environments, most contamination is brought in by human activities. In this case, it has been shown that most of the biocontamination could have been carried by skin fragments between 4 and 20 µm. Moreover, the quality of indoor air in terms of its bioaerosol is important due to its potential aetiological role in development of conditions such as sick building syndrome. It may help to elucidate the role of viable particles in disease states, particularly allergy-related conditions. This justifies our study, which correlates the concentration in deposited macroparticles (size >5 µm) with the viable particles. In addition, only a small fraction of microorganisms collected is viable. Today, there are reliable resources which are adapted to measure continuously macroparticles from 5 µm (CLEAPART-100 in this study). We have shown that the deposited macroparticles results could be strongly correlated to viable particle measurements (bacteria and mold) after dynamic sampling by biocollectors. The difficulty is that viable particle measurements require an incubation period of at least 48 h to glow the viable particles to a size which may be seen by the eye. We can then understand the advantage in macroparticle (viable and nonviable) monitoring with a continuous detector such as the CLEAPART-100 which detects all contamination events earlier. The CLEAPART-100 is therefore an instrument which enables the alert to be raised in relation to any contamination

or biocontamination events. In this study, we have also shown that less than 1% of particles were viable particles, which conforms to the data in the literature and which justifies carrying out the double measurement in parallel for the viable microorganisms and the total macroparticles. We also obtained a low dispersal between the results obtained using three different biocollectors, which can be explained by the same sampling rate for the three biocollectors and a strictly identical sampling and incubation protocol for each of them. In the future, we intend to use the CLEAPART-100 in clean-room such as in hospitals or pharmaceutical environments as it helps controlling bacterial multiplication at critical points in controlled environments.

Acknowledgments

This research is supported by CEA and Winlight System.

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