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Some aspects on the sampling efficiency of microbial impaction air samplers

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ABSTRACT

Indoor microbial monitoring is an important health issue in many sectors of society. In particular, it is important to monitor microbial concentrations in environments dealing with bio-susceptible products. Many human diseases are related to high, undesired microbial airborne concentrations. However, the lack of a standardized and well-accepted methodology for testing and ranking the performance of microbial air samplers is a source of uncertainty in such measurements. Several works clearly show that results obtained from microbial air sampling depend largely on measuring techniques, especially the air samplers' physical parameters, such as d_{50}, as well as environmental conditions, sources, and concentrations of microbial organisms in the environment. Furthermore, personnel using cleanroom clothing can reduce the microbial burden within a clean environment. To evaluate this effect, we carried out experimental comparison tests in a cleanroom of class ISO 5 with different air samplers under various microbial concentration levels, generated by a human source dressed in different quality cleanroom clothing. Our results confirm that in addition to the measuring technique, cleanroom clothing does influence microbial contamination, affecting air sampler measurements.

Introduction

In many industrial and civil settings, accurate monitoring of bioaerosol concentrations is important for maintaining product quality and human health (Lee et al., 2004; Saldanha, Manno, Saleh, Ewaze, & Scott, 2008). In special cases, such as in pharmaceutical, dairy and other food industries, as well as hospitals, such monitoring of the microbial airborne concentration is required to prevent human diseases (Kruppa & Rüden, 1996; Napoli et al., 2012). There is also growing interest in bioaerosol concentrations in common indoor spaces, such as office buildings, domestic buildings, and schools because of their effect on human health (Frankel, Timm, Hansen, & Madsen, 2012; Tsai & Macher, 2005). Monitoring viable airborne particles is a specific form of aerosol measurement. Generally, bioaerosol particles range in size from 0.001 to 100 μm (Baron & Willeke, 2001). They are collected using air samplers, but identified and quantified by growing them on suitable culture media, such that they form visible clusters or colonies (Roszak & Colwell, 1987). Concentration is usually expressed in terms of the colony forming unit per cubic meter of sampled air (CFU/m^3). Although many air samplers rely on the same technological principles, their results are not comparable. It is not unusual to have different microbial concentrations from different air samplers that have sampled the same environment (Frankel et al., 2012; Willeke, 1976). Thus, sampling performance depends on many factors, including variations in environmental conditions, bioaerosol source and load, as well as instrument-related features (Baron & Willeke, 2001; Seinfeld, 1986; Zhen et al., 2009). Moreover, experimental tests show discrepancy with field tests, confirming the variability of such measurements. This is also compounded by the absence of a well-accepted standard protocol for sampling bioaerosols (Saldanha et al., 2008; Willeke, 1976). This makes the choice of air sampler for a monitoring task, and the interpretation of its results, an important but difficult issue. Although ISO Standard 14698-1 (2003) Annex B describes a way to assess the microbiological sampling of air samplers, it does not provide a standard air sampling protocol.

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Table 1
Calculated d_{50} values for samplers using Eq. (1) (after Nevalainen et al. (1993)) and simplified Eq. (2) (after Meier & Zingre, 2000).

Sampler	Flow rate (L/min)	Velocity (m/s)	d_{50}[a] (μm)	d_{50}[b] (μm)
A	100	10.85	1.61	1.62
B	50	29.76	1.64	1.65
Stage No. of C				
1	28.3	0.86	7.84	7.91
2		1.53	5.09	5.13
3		2.42	3.61	3.64
4		4.04	2.46	2.48
5		12.77	1.04	1.05
6		64.64	0.31	0.31
D	100	n.a.[c]	n.a.	n.a.
E	28.3	1.24	14.31	14.44

[a] Calculated according to Eq. (1).
[b] Calculated according to Eq. (2).
[c] n.a.: not applicable.

An experimental study (Ljungqvist & Reinmüller, 1998) compared a selection of air samplers currently used for monitoring and demonstrated that some samplers collected up to 10 times lower CFUs than others. This shows that when low-efficiency samplers are used, a non-compliant environment can erroneously appear to achieve an acceptable indoor air quality. Similar conclusions were reached in indoor assessments, which used different samplers (Frankel et al., 2012). An extensive field comparison of four air samplers employed in public buildings (Lee et al., 2004) showed that the concentration of bioaerosols, i.e., mold, was highly dependent on the specific characteristic of the samplers and the analytical method used for a specific environment. Some differences in sampling bioaerosols are related to their inherent peculiarities. Their different origins result in different characteristics, such as size, shape, and density, which in turn determine their behavior as airborne particles. For example, the motion of a particle affects the efficiency of impaction devices (e.g., slit-to-agar samplers, sieve samplers, centrifugal samplers) and settling plates, commonly used for air sampling. Since the behavior of an airborne particle within these sampling devices is governed by the aerodynamic flow around the particle, it depends on particle size, measured in terms of the particle's aerodynamic diameter. Thus, d_{50} (cut-off size) describes the aerodynamic or equivalent particle diameter for which 50% are removed from the air stream and collected.

In this study, we evaluate the performance of four different microbial air samplers, subjected to different microbial loads, generated by a human source. Since microbial concentrations generated by humans vary with the cleanroom clothing system used and the behavior adopted, if these factors are not controlled, they affect CFU/m^3 concentrations in cleanroom environments. In this context, the choice of a low-penetration fabric for cleanroom clothing and a suitable d_{50} may improve the performance of different air samplers.

Methods

A comparative study with three impaction air samplers and one gelatin filter sampler was carried out in a controlled environment, corresponding to an ISO class 5 cleanroom (particles ≥0.5 μm), as described in ISO 14644-1 (1999). Air samplers evaluated during our experimental tests included a sieve impactor (Sampler A with 400 holes of 0.7 mm), a slit-to-agar sampler (Sampler B with a slit of 28 mm in length and 1 mm width), a six-stage sieve impactor (Sampler C), and a membrane filter (Sampler D with pore size of 3 μm). A second sieve impactor (Sampler E with 12 holes of 6.35 mm) was also considered in this work. The d_{50} (μm) for each of these air samplers was calculated using (Hinds, 1999; Nevalainen et al., 1993):

$$d_{50} = \sqrt{\frac{9\eta D_h Stk_{50}}{\rho U C}}\, 10^6, \quad (1)$$

where η = viscosity of air (Pa·s); D_h = hydraulic diameter of the air inlet nozzle (m); Stk_{50} = Stokes number that gives the 50% collection efficiency (non-dimensional); ρ = particle density (kg/m^3); U = impact velocity (m/s); and C = Cunningham correction factor used for particles smaller than 1 μm (non-dimensional). Eq. (1) is simplified using constant factors for air viscosity, particle density, and the correction factor (Ljungqvist & Reinmüller, 2008; Meier & Zingre, 2000). Thus, the expression for d_{50} (μm) becomes:

$$d \approx \sqrt{\frac{40 D_h 1000}{U}}. \quad (2)$$

Table 1 gives the characteristics of each air sampler, and their d_{50} values calculated using Eqs. (1) and (2).

The three impaction air samplers (Samplers A, B, and C) all had d_{50} values below 2 μm, Sampler E had d_{50} value below 15 μm, while Sampler D, the gelatin filter, had a pore size below 3 μm. All air samplers were operated according to the manufacturers' instructions. High efficiency particulate air (HEPA)-filtered air was introduced into the test chamber with an air velocity of ca. 0.4 m/s. All air samplers were tested simultaneously under the same environmental conditions. The only source of contamination was a person, dressed in one of the three different clothing systems, walking in the air stream in front of the measuring device for a period of 10 min. Three different clothing systems were used to generate three different bio-contamination levels, referred to here as high, medium, and low. Each test was repeated three times at each contamination level, while air samplers were sampling continuously. Our experimental layout is shown in Fig. 1.

The microbial growth medium used for Samplers A, B, C and E was the standard TSA (tryptic soy agar) in 9-cm Petri dishes, while gelatin filters from Sampler D were placed on a sterile blood agar plate. All sampling plates were incubated for at least 3 days at 32 °C, and then 2 days at room temperature. All measurements are given in terms of CFU/m^3.

Results and discussion

Concentrations detected by each device for the three concentration levels assessed are shown in Table 2. The results show that CFUs for all samplers had similar ranges, even though a small difference occurred between Sampler A and D. The lowest CFU concentrations were measured by Sampler D. Despite their identical d_{50} value,