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Sampling methods for an accurate mycobiota occupational exposure assessment—overview of several ongoing projects

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ABSTRACT: Exposure to bioaerosols is a critical occupational issue that requires close attention. Workers in several occupational environments, such as health care, agriculture, animal production, waste, fishery, forestry, mining, construction, and day care are exposed to higher risks of biological hazards due to work characteristics. This review intends to provide information on what is currently known about sampling methods to achieve mycobiota exposure assessment, since fungal burden characterization continuous to be a challenging task for every industrial hygienist. A brief description about Research Group Environment & Health (GIAS) contribution on this topic is also given with an overview of the developed and ongoing projects. Passive and active methods should be applied in parallel to ensure a more precise occupational exposure assessment to the fungal contamination. Increasing the number of different sampling methods will enrich data findings enabling industrial hygienists to perform risk characterization.

1 INTRODUCTION

Airborne microorganisms might pose an occupational hazard when present in high concentrations in occupational environments resulting in health problems (Stetzenbach, Buttner & Cruz 2004). The presence of high levels of bioaerosols, and more specifically the mycobiota content, is frequently the result of the natural colonization of an organic substrate present in the workstation but may also be intentionally added (the case of food industry) (Oplliger 2014). Therefore, exposure to bioaerosols is a critical occupational issue that requires close attention (Wang et al. 2015). The workers in different settings, such as health care, agriculture, animal production, waste, fishery, forestry, mining, construction, and day care are exposed to higher risks of biological hazards because of the work characteristics (Wang et al. 2015; Viegas et al. 2015a). Numerous studies have indicated that these workers have higher prevalence rates of respiratory diseases and airway inflammation (Heldal et al. 2003; Bang et al. 2005; Heederik et al. 2007; Cox-Ganser et al. 2009).

Of note, is the uniquely of each bioaerosol sample as its composition varies in time and space (abundance and diversity of species) (Oppliger 2014). Thus, exposure assessment to bioaerosols and more specifically to the mycological content, remains to be a challenging task for every industrial hygienist. Occupational exposure to microbiological risks can be estimated using a variety of different methods and each situation is unique and requires specific methodology (Oppliger 2014; Viegas et al. 2015a).

Information about on what is currently known concerning sampling methods to achieve mycobiota exposure assessment will be provided. In addition, a brief description about Research Group Environment & Health (GIAS) contribution on this topic is also given with an overview of the developed and ongoing projects.

2 SAMPLING METHODS

2.1 *Active methods*

The active methods devices used to sample airborne fungi mainly rely on three different principles namely, impaction, impingement and filtration (Mandal & Brandl 2011; Viegas et al. 2015a). Impactors use solid media such as agar to collect bioaerosols by impactation. Their easiness to handle and the cheap cost are major advantages (Zollinger, Krebs & Brandl, 2006; Viegas et al. 2015a). The colonies number can be counted by visual inspection after incubation resulting in a direct quantitative estimate of the number of culturable fungi in the volume of air sampled

(Zollinger, Krebs & Brandl 2006; Viegas et al. 2015a). This method is the chosen by ACGIH (Verhoeff, Van Wijnen & Brunekreef 1992) and recommended by the Canadian Health Organization (Health Canada 1993).

In impingers particle collection is based on liquid media. Normally, sampled air is drawn by suction through a narrow inlet tube into a small flask containing the collection medium. Once the sampling is complete, aliquots of the collection liquid can be cultivated in appropriate growth media to enumerate viable microorganisms allowing quantitative determinations, since the sample volumes and sampling times can be previous defined (Zollinger, Krebs & Brandl 2006; Viegas et al. 2015a). Formerly known as scrubbers, they are often necessary in occupational settings with higher fungal loads. This approach, besides allowing dilution of the sample prior to plate incubation, also easier the application of molecular tools since a liquid air sample is expected after the sampling. Both these features are not generally possible with samplers that employ impaction on solid media (Thorne & Heederik 1999; Viegas et al. 2015a). On the downside, fungi present in small numbers and as single units may be less represented (Macher 2001) and impingers cannot operate for long periods since liquid evaporation can hamper the fungi viability (De Nuntiis et al. 2003).

Filtration samplers collect particles air through suction filters. Air is drawn by a vacuum line through a membrane filter that can be made of glass fibre, Polyvinylchloride (PVC), polycarbonate or cellulose acetate or gelatin (Mandal & Brandl 2011). The filter membrane can be placed on a culture media and incubated to allow fungal growth or even digested with a tampon solution such as sterile Phosphate Buffered Saline (PBS) and then inoculated in the selected media (Sudharsanam et al. 2012; Viegas et al. 2015a). Filter samples can also be dispersed in a liquid prior to cultivation enabling higher colony counts (Viegas et al. 2015) and a more straightforward bench work in case of applying molecular tools (Viegas et al. 2015a). Due to the risk of dehydration—since the surface where the particles are collected is completely dry—this method is only suited for resistant microorganisms, such as fungal spores (De Nuntiis et al. 2003).

Personal sampling is the ideal method for assessing personal bioaerosol exposure (Wang et al. 2015). An perfect personal bioaerosol sampler used in occupational environments should be light and robust, noninterfering with the work tasks, able to collect selected bioaerosols, and with suitable biological sampling efficiencies (Agranovski et al. 2002). The filtration method is the one generally applied for personal bioaerosol exposure assessment.

2.2 *Passive methods*

Passive sampling provides a valid risk assessment as it measures the harmful part of the airborne population which falls onto a critical surface (French et al. 1980). In less contaminated occupational environments, such as hospital facilities, passive monitoring uses mainly "settle plates", which are standard Petri dishes. These plates contain culture media exposed to the air for a given time in order to collect biological particles which "sediment" out and are then incubated. Results are expressed in CFU/plate/time or in CFU/m³/hour (Pasquarella, Pitzurra & Savino 2000). This method was already reported as the only method applied to ensure the bioburden exposure assessment (Hayleeyesus & Manaye 2014).

In settings with higher mycobiota burden the trend is to complement active methods with different passive methods such as surface swab (Viegas et al. 2016c) and, more recently, Electrostatic Dust Cloth (EDC) (Normand et al. 2009; Viegas et al. 2017c).

Surface swabs complement microbiological characterization of the air and are used in order to identify contamination sources and to evaluate efficacy of surface cleaning and disinfection procedures (Klánová & Hollerová 2003; Stetzenbach, Buttner & Cruz 2004; Viegas et al. 2016c). Surface samples are collect by swabbing the surfaces using a 10×10 cm² stencil disinfected with 70% alcohol solution between samples according to the International Standard ISO 18593 (2004). Specifically for the mycobiota assessment surface swabs already showed higher diversity in terms of the number of fungal species detected, as well as a higher fungal load, when compared with the air samples proving the relevance of analysis of the latter samples in complementing the results obtained by air sampling (Viegas et al. 2016c).

The EDC is an easy-to-use passive device that consists on a polypropylene cloth (Kilburg-Basnyat et al. 2016) and is increasingly being used because it is electrostatic, inexpensive, easy to obtain, and effective at collecting dust (Cozen et al. 2008). EDCs employ electric fibers which have revealed to increase particle retention (Kilburg-Basnyat et al. 2016). Despite not widely used in occupational exposure assessments the main advantage from EDC is that they can collect contamination from a larger period of time (weeks to several months) as in the analyzed papers, whereas air samples can only reflect the load from a shorter period of time (mostly minutes) (Viegas et al. 2015a; Viegas et al. 2017c).

The importance to employ, in parallel, active methods and passive methods was already reported (Brenier-Pinchart et al. 2009; Viegas et al. 2016c; Viegas et al. 2017c) since active methods provide information about the contamination load, while using passive methods such as surface swabs and EDCs gives us a more detailed scenario regarding occupational exposure to mycobiota (Viegas et al. 2017c).

Of note, depending on each occupational environment the assessment can be complemented with different environmental matrices, such as litter and feed in animal production (Sabino et al. 2012; Viegas et al. 2012; Viegas et al. 2013), air conditioning filters from fork lifters managing waste (Viegas et al. 2017b), raw materials on feed industry (Viegas et al. 2016a) settled dust in bakeries (data not available). The obtained results besides providing information about the contamination sources can also enrich exposure assessment with data and, consequently, the risk characterization.

Table 1. Sampling methods applied from the different projects developed and ongoing on GIAS.

Occupational setting	Active methods	Passive methods	Sampling and analyses methods	Reference
Poultries	Impaction Impinger	Surface swabs	Impaction and surface swabs: Culture based methods	Viegas et al. 2014c
WWTP	Impaction Impinger	Surface swabs		Viegas et al. 2014a
WTP	Impaction Impinger	Surface swabs	Impinger: Molecular tools	Viegas et al. 2014b
Cork Industry	Impaction Impinger	Surface swabs		Viegas et al. 2015b
Feed Industry	Impaction Impinger	Surface swabs		Viegas et al. 2016a
Slaughterhouses	Impaction Impinger	Surface swabs		Viegas et al. 2016b
Swine (on going)	Impaction Impinger	Surface swabs	Impaction: Culture based methods Impinger and surface swabs: Molecular tools	-
Bakeries (ongoing)	Impaction Impinger Filtration	Surface swabs EDC	Impaction: Culture based methods	-
Hospital facilities (starting)	Impaction Impinger Filtration	Surface swabs EDC	All the other sampling methods applied culture based-methods and molecular tools	-

3 PROJECTS OVERVIEW

Since 2010 the Research Group Environment & Health (GIAS) from Escola Superior de Tecnologia da Saúde de Lisboa, Instituto Politécnico de Lisboa, has been focusing on the mycobiota occupational exposure assessment in different occupational environments (Poultries, Waste Water Treatment Plants—WWTP, Waste Treatment Plants—WTP, Cork industry, Feed industry, Slaughterhouses, Swine, Bakeries and Hospital facilities) (Table 1). Active and passive methods have being applied to pursuit the most suitable and accurate fungal contamination exposure assessment with an increase of sampling methods, and also analyses, performed through the years.

No significant correlation was found on fungal loads obtained through both active and passive samplings (Viegas et al. 2014–2017) as in other study (Petti, Iannazzo & Tarsitani 2003). The lack of correlation can be justified by a wide range of environmental variables, such as workers who may carry the mycobiota indoors (Scheff et al. 2000), as well as the developed activities and work practices that may affect fungal load (Jürgensen and Madsen 2016). Furthermore, when correlation is tested only with culture base methods results, we cannot disregard also the fact that viable fungi constitute a small percentage of the total concentration of the mycobiota (Huang et al. 2013; Viegas et al. 2015a) and, therefore, a bias about the fungal load is expected.

4 CONCLUSIONS

Passive and active methods should be applied in parallel to ensure a more precise occupational exposure assessment to the fungal contamination. Increasing the number of different sampling methods will enrich data findings, enabling industrial hygienists to perform risk characterization.

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