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A critical review of analysis techniques for the assessment of the indoor fungal burden

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Abstract

The assessment of indoor fungal growth has attracted the attention of the research community for many decades. In the effort to assess the fungal burden in the built environment, multiple analysis techniques have been established and offer a plethora of information for the extent of contamination and fungal diversity. However, all analysis techniques are accompanied by drawbacks and the selection of the most appropriate method can become challenging for researchers and practitioners. The aim of this study is to present the most widely used analysis techniques, underline their merits and disadvantages, and provide guidance on the selection process to ensure that the analysis outcomes match the aims of the investigations.
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Keywords: fungi, mould, culture, PCR, biochemical, immunochemical

1. Introduction/Background

The selection of the most appropriate analysis method for the processing of indoor air samples has been within the researchers' scope of interest for more than half a century. However, though numerous methods exist, concerns regarding the most suitable combination of air sampling and analysis techniques and the reliability and accuracy of the testing results are still baffling researchers and investigators working on indoor fungal testing. This study aims to identify the most widely used analysis techniques, showcase their merits and drawbacks, and discuss how the selection process should reflect the aims of the investigation.

2. Critical review of analysis techniques

The analysis methods for air samples are often classified into culture-based and non-culture-based methods depending on whether fungal growth is necessary for the processing of the samples or not [1]. However, though numerous techniques are currently available, only a few have been widely used in literature. This study aims to summarize the most common analysis techniques found in recent standards and research papers and determine their key characteristics.

Culture-based method/colony enumeration: Culture-based methods involve the cultivation of culturable fungal spores recovered during sampling, on culture media under specific conditions. They can be easily used but they depend highly to the conditions of sampling, incubation and the culture media [2].

Microscopy/ Flow cytometry: Both microscopy and flow cytometry are used to obtain cell information optically [3]. Microscopy can be used to monitor cells over time. Flow cytometry, on the other hand, allows rapid analysis of multiple cells' characteristics but cannot be used to monitor cells with time [3].

Polymerase chain reaction (PCR): PCR methods involve the amplification of nucleic acids recovered during sampling. Whether full identification of the fungal species present on the sample, is achieved, depends on the PCR method selected. For example, conventional PCR provides qualitative results, while real-time PCR (qPCR) allows the quantification of starting sequences. The selection of advanced PCR methods is often limited by the availability and cost of specialized equipment [4].

Biochemical and immunochemical assays targeting:

a) **β-glucans**: Tests measuring b-glucans can be used to indicate health risks from exposure to the air of the space sampled [5]. Commonly used tests for the determination of β glucans are the Limulus amebocyte lysate (LAL) assay and Enzyme-Linked ImmunoSorbent Assay (ELISA) [6]. Though these methods can be used as a proxy for pathogenic potential, the fact that only partial identification of fungal species can be achieved makes them unsuitable for the assessment of risks related to structural integrity.
b) **Ergosterol:** Methods targeting ergosterol have been utilized in various studies to estimate fungal biomass [7-9]. However, the accuracy of the results may be subject to phenomena that are not yet totally clear —e.g. change in the ergosterol production rate due to physiological changes of the mycelium with age or the substrate material [9].

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c) **Adenosine Triphosphate (ATP):** Assays targeting ATP can be used to assess the activity of microorganisms in the indoor environment sampled. Considering that ATP can be produced by various sources the estimation of fungal biomass by these methods may not be accurate and cannot indicate the pathogenic potential of the environment samples.
d) **Extracellular enzymes (NAHA**): Methods targeting extracellular enzymes (NAHA) can allow the estimation of the indoor fungal burden [10]. However, NAHA may also be produced by certain bacteria, protozoa and mammalian cells, hence, could potentially lead to overestimation of the fungal burden [11].
e) **Chemical bi-products:** Methods targeting chemical bi-products rely on the collection of data through sampling devices that are used for the assessment of indoor air quality. They can be used to indicate potential health risks posed by exposure to the environment under assessment but cannot provide information for the estimation of fungal biomass [12].

Discussion

While the literature has been dominated by culture-based methods until the early 2000s, the rapid developments in molecular biology have influenced many researchers and practitioners into shifting to DNA sequencing, and chemical and immunochemical assays targeting fungal cell constituents and extracellular compounds for the analysis of indoor air samples. The review demonstrates that the analysis techniques are connected to the aims of the investigation. While culture-based methods can be utilized to assess the amount of culturable airborne fungi, they cannot allow the estimation of the total fungal biomass (viable and non-viable fungi) in a room. On the other hand, LAL assays may allow the estimation of the fungal burden but cannot be used for investigations assessing the fungal particles' viability. An appropriate analysis method should maximize the testing outcomes' accuracy and effectively support the role of the investigation (estimation of the fungal biomass, assessment of indoor pathogenic potential, assessment of indoor fungal viability, etc.)

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