Indoor fungal contamination: Health risks and measurement methods in hospitals, homes and workplaces

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Abstract

Indoor fungal contamination has been associated with a wide range of adverse health effects, including infectious diseases, toxic effects and allergies. The diversity of fungi contributes to the complex role that they play in indoor environments and human diseases. Molds have a major impact on public health, and can cause different consequences in hospitals, homes and workplaces. This review presents the methods used to assess fungal contamination in these various environments, and discusses advantages and disadvantages for each method in consideration with different health risks. Air, dust and surface sampling strategies are compared, as well as the limits of various methods are used to detect and quantify fungal particles and fungal compounds. In addition to conventional microscopic and culture approaches, more recent chemical, immunoassay and polymerase chain reaction (PCR)-based methods are described. This article also identifies common needs for future multidisciplinary research and development projects in this field, with specific interests on viable fungi and fungal fragment detections. The determination of fungal load and the detection of species in environmental samples greatly depend on the strategy of sampling and analysis. Quantitative PCR was found useful to identify associations between specific fungi and common diseases. The next-generation sequencing methods may afford new perspectives in this area.

Introduction

For some years, concern about human exposure to microorganisms in indoor environments has been focused on fungi. Interest in bioaerosol exposure has increased significantly because it is now recognized that exposure to fungal agents is associated with a wide range of adverse health effects with a major impact on public health. As people in modern society spend 80% or more of their time indoors (Bernstein et al., 2008), many methods have been developed to assess indoor fungal exposure.

Various indoor environments are concerned by fungal health risk. Improved insulation of buildings combined with poor ventilation has generated environments that favor the growth of mold and several studies have suggested that a significant proportion of the occurrence of building-related diseases is associated with exposure to mold (Bornehag et al., 2004; Crook & Burton, 2010; Douwes et al., 2003; Reboux et al., 2010; Singh, 2001). According to the World Health Organization guidelines on indoor air quality (OMS, 2009), occupants of damp or moldy buildings have up to 75% greater risk of respiratory symptoms and asthma. Significant fungal exposure also occurs in agricultural and industrial activities and may cause occupational respiratory diseases (Reboux et al., 2006). In hospitals, the prevention of nosocomial fungal infections is currently based on air-control measures with monitoring fungal contamination (Gangneux et al., 2006a).

Fungi can affect human health in a variety of ways such as infections, allergic reactions (sensitization and immune over-reaction), irritations and toxic reactions (Fischer & Dott, 2003; McGinnis, 2004). Fungal infections range from superficial to invasive infections. For example, invasive aspergillosis is not only a life-threatening infection in neutropenic patients but also occurs in patients with chronic pulmonary diseases and/or undergoing corticosteroid treatment (Sherif & Segal, 2010; Thornton, 2010). Exposure to mold can cause allergic reactions in fungal-sensitive individuals, who account for about 10% of the total population and 40% of patients with asthma (Burge, 2001; Mendell et al., 2011). Toxic reactions are mainly caused by mycotoxins which are secondary fungal metabolites (Jarvis & Miller, 2005; Nielsen, 2003) but components of fungal cell wall, such as (1→3)-B-D-glucans (Douwes, 2005), have also been reported as having a causal role. In addition, exposure to volatile organic compounds (VOCs) produced by fungi growing on and degrading substrates may be responsible for
nonspecific symptoms, such as headaches, eye, nose and throat irritations and fatigue (Wältinder et al., 2005). This wide range of chemical by-products is partly carried in indoor air by fungal fragments and spores. The biological mechanisms as well as the causative factors related to the effects of fungi on human health remain nevertheless unclear.

To deal with the complexity of the diverse effects of mold on health, many measurement strategies have been developed to assess indoor fungal contamination. Although hospitals are very different from home and agricultural environments (in terms of fungal contamination and biodiversity), there may be similarities in sampling and analysis methods. In this article, an overview is provided on the health effects associated with fungal exposure in these three environments. In addition, a comprehensive description of fungal exposure assessment methods used over the last decade is presented. Advantages and disadvantages for each method are presented in consideration with different health risks in hospital, home and workplaces. This review also identifies technical challenges in fungal measurement, with specific interests on viable fungi and fungal fragment detections. Finally, the potential use of enhanced fungal monitoring with the next-generation sequencing methods is discussed.

Environmental and public-health issues

Control of fungal environmental risk in hospitals

Fungal infections are a public health issue in hospitals. Aspergillosis is the most significant opportunistic disease in immunocompromised patients: this fungal infection is caused primarily by *Aspergillus fumigatus*, but also to a less degree by *Aspergillus flavus*, *Aspergillus nidulans*, *Aspergillus niger* and *Aspergillus terreus* (Garcia-Vidal et al., 2008; Perfect et al., 2001). Other fungi causing respiratory infections include *Acremonium*, *Paecilomyces*, *Rhizopus*, *Mucor*, *Absidia* and *Fusarium* (Fleming et al., 2002; Lanternier et al., 2012). Exposure to these environmental fungi occurs through inhalation of air (Peláez et al., 2012) or aerosolized droplets of water, e.g. during a bath or shower (Anaisie et al., 2002; Warris & Verweij, 2005). Despite improvements in diagnosis and treatment, invasive fungal infections are often fatal; the mortality rate remaining around 50% for invasive aspergillosis (Lortholary et al., 2011).

Preventive measures are, therefore, taken in operating rooms and in high risk units. In particular, high efficiency particulate air (HEPA) filtration systems and rooms with laminar airflow are needed in hematology wards to minimize ambient fungal spore concentrations (Araujo et al., 2008; Faure et al., 2002). An environmental surveillance program is often implemented in hospitals in order to eliminate opportunistic fungi detected in these high risk areas as rapidly as possible (Alberti et al., 2001; Chang et al., 2008; Morrison et al., 2004). Although the strategies developed to prevent invasive fungal infections are costly, these are widely adopted given the high cost of diagnosing and treating these often fatal diseases. Specific protective measures against contamination must be taken when building work is being carried out on hospital sites, such as keeping rooms, ward doors and windows closed, reinforcing disinfection and using air treatment systems (Fournel et al., 2010; Gangneux et al., 2012).

Detection of abnormal mold development at home

Indoor fungi in housing may cause adverse effects, both on buildings and residents. In addition to damaging materials in the home, they can render a building unpleasant to live in by looking and smelling bad (Portnoy et al., 2004). Dampness and molds in buildings are associated with increases of 30–50% in a variety of respiratory disorders (Fisk et al., 2007), and are determinants of developing asthma (Quanah et al., 2012). It was recently shown that exposure during infancy to three mold species common to water-damaged buildings (*Aspergillus ochraceus*, *Aspergillus ungeri* and *Penicillium variabile*) was associated with childhood asthma at age 7 years (Reponen et al., 2012). Indoor fungi may also be an important factor in the development of sick building syndrome (Allermann et al., 2006; Crook & Burton, 2010; Takigawa et al., 2009). Detection of abnormal fungal development in homes is essential not only to assess the exposure of atopic individuals predisposed to respiratory diseases, but also of non-atopic individuals. Detection of viable fungi responsible for invasive fungal infection in dwellings is useful for assessing the risks to immunosuppressed patients at home, because an increasing number of chemotherapy protocols have a short-stay, and so the immune system is not completely restored on release from hospital.

The fungal diversity in homes is high, but *Cladosporium*, *Alternaria*, *Aspergillus* and *Penicillium* species are frequently found in this environment. The main mold growth factors in dwellings are water infiltration or leakage, poor insulation, thermal bridges, condensation and lack of ventilation.

Fungal exposure has been assessed by questionnaire in numerous studies. However, this method is likely to give biased results, especially when inhabitants with respiratory problems fill in the documents (Burr, 2001; Fisk et al., 2007; Ren et al., 2001). In many studies, questionnaires are administered by trained interviewers who also report dampness signs and visible molds. However, these criteria are not necessarily sufficient, because fungal development may be hidden in building materials or ventilation systems. Objective and quantitative tools are, therefore, required to assess exposure to molds in homes.

Assessment of the fungal risk in the workplace

There is growing public awareness regarding the risk associated with poor indoor air quality in the workplaces. Exposure at work to dampness and molds was associated with the occurrence of new-onset asthma (Karvala et al., 2011). In agricultural environments, the respiratory system is particularly exposed to many inorganic and organic agents which may cause or exacerbate asthma, mucous membrane irritation and chronic bronchitis (Adhikari et al., 2004b; Cormier et al., 2000; Danuser et al., 2001; Omland, 2002; Radon et al., 2002). Farmers are known to be at high risk of developing occupational airway disease. Common molds such as *Absidia corymbifera*, *Eurotium amstelodami* and *Wallula sebi* are implicated in farmer’s lung disease, the most common form of occupational hypersensitivity pneumonitis (Reboux et al., 2001). Occupational airway disease can occur in many different environments and so a wide variety of fungi may be implicated (Karvala et al., 2008). For example,
bioaerosols released by the waste recycling and composting industry are a recent cause of concern because of their potential impact on the health of workers (Bünger et al., 2007; Fischer & Dott, 2003; O’Gorman, 2011).

In these workplaces, exposure to mycotoxins is mostly not only by ingestion, but also occurs by the dermal and inhalation routes. Some mycotoxins are known carcinogens (ochratoxin, aflatoxin, etc.) that may, for example, be inhaled in food and animal feed industries (Jarvis & Miller, 2005). High spore concentrations of Wallemia sebi, known to produce a toxic metabolite (wallemolin A), may also be found in hay and grain storage facilities and stabling (Zeng et al., 2004). However, little is known about inhalation and limits for the concentration of airborne mycotoxins and these compounds are rarely monitored in these environments.

Airborne fungal concentrations in farming environments are not easily measured owing to very high concentrations ($10^3$–$10^{10}$ CFU/m$^3$). An exposure level of about $10^5$ spores/m$^3$ has nevertheless been associated with respiratory troubles (Eduard, 2009). A microbiological assessment of the environment contributes to the diagnosis and treatment of occupational airway diseases by identifying both the circumstances of exposure and the sources involved (Roussel et al., 2006).

Indoor fungal contamination is a public health concern that may affect various populations (patients, residents, workers) depending on the context and the individual susceptibility. There are many adverse health effects with differing degrees of severity, and the development of some diseases is known to be associated with specific molds. Fungal concentration and diversity depend on the specific characteristics of the indoor environment and so the aim of sampling for fungi may vary, as well as the techniques used to detect fungal contamination.

Environmental sampling techniques

The main reason for collecting samples when fungal contamination is suspected is to detect, quantify and identify any fungi that might be present (Portnoy et al., 2004). Before starting a survey, certain essential factors should be established and evaluated: the reasons for undertaking the inspection, the types of fungi being looked for, the sampling frequency and the places to be sampled. In hospitals, samples are usually taken in areas with air-treatment systems where the presumed level of aerobic contamination is very low (such as wards with high efficiency filtration and operating rooms) (Gangneux et al., 2002). In housing studies, the places where samples are taken depends either on the time spent in a room (bedroom or living room) or on the presence of high humidity (bathroom or kitchen) (Jovanovic et al., 2004; Reboux et al., 2009; Ren et al., 2001). In workplaces, the scope of the sampling strategy should take account of the symptoms of the workers and their occupations.

Air sampling

As molds are associated with diverse respiratory disorders, air sampling is often performed in indoor environments (Table 1). For example, it is useful for determining whether the air in homes or workplaces is microbiologically normal or atypical, by comparison with data from other groups of homes or workplaces. Air is also sampled in hospitals as part of surveillance programs. Bioaerosol sampling may be carried out by impaction onto a culture plate inserted into the sampling device or by impingement into a liquid medium. Impaction sampling durations of 1–10 min are appropriate for avoiding media desiccation (Godish & Godish, 2007). The use of impingers is a flexible method for producing samples for a range of laboratory techniques for analysis. Pre-sterilized cassettes with filters can be used for personal or area air sampling, especially in working places to assess individual exposure. Filtration methods allow long-term measurements and are applicable during normal activities in the room and when major air movements and fluctuating mold concentrations are to be expected. Sampling volumes are usually adapted to the level of fungal concentration. They are, therefore, higher in hospitals (usually 500 L or 1 m$^3$) with low presumed levels of biocontamination than in workplaces where lower volumes will avoid saturating the sample by impaction (usually <300 L) (Table 1). Sampling conditions (air flow rate and sampling time) vary hugely depending on the type of sampler and the environment. The use of high-volume centrifugal or cyclonic samplers is increasing: They often operate at greater than 300 L min$^{-1}$. The design of the air samplers is known to influence their efficiencies for measuring airborne microbial concentrations (Yao & Mainelis, 2007). As limited information is usually available about their overall performance, comparisons of different bioaerosol samplers in field conditions is useful to assess their performance (An et al., 2004; Gangneux et al., 2006b; Haatainen et al., 2010).

Surface sampling

Surface sampling can determine whether a mark on a wall has resulted from fungal growth or some other cause. It can also assess the effectiveness of remediation and clean-up of indoor environments. Direct microscopic analysis of tape lift samples can provide a semi-quantitative determination of the genera and number of spores present (Barnes et al., 2010; Boutin-Forzano et al., 2004). A cotton swab can also be used for subsequent culture and molecular analyses (Cai et al., 2009; Reboux et al., 2009). In homes, surface samples are obviously positive if they are taken from moldy areas but can be used to determine the fungal diversity of the sampled areas. In hospitals, surfaces are sampled in addition to air sampling, in order to optimize the sensitivity of surveillance in areas that are presumed to have low contamination. The method must be standardized. It has been proposed, for example, that 5 cm $\times$ 5 cm areas should be sampled systematically on various sites (basin, air extraction grid, television, etc.), in order to determine the contamination kinetics during long-term surveillances (Gangneux et al., 2006a). Direct application of culture media is also a common method in hospitals (Faure et al., 2002; Sixt et al., 2007).

Dust sampling

Dust can be analyzed to determine the presence of fungi or fungal agents that have accumulated over time, as it provides an indication of the microbial agents that may have been airborne (Table 2). The term “settled dust” is often used to...
<table>
<thead>
<tr>
<th>Air sampler</th>
<th>Volume range (L)</th>
<th>Hospital</th>
<th>Home</th>
<th>Workplace</th>
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<tbody>
<tr>
<td>Impactor</td>
<td>&lt;100</td>
<td>Wu et al. (2000)</td>
<td>Ren et al. (2001)</td>
<td>25 Lpm 1–2 min</td>
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<td></td>
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<td>Fabian et al. (2005); Lee &amp; Jo (2006)</td>
<td>28.3 Lpm 0.5 min</td>
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<td>O’Connor et al. (2004); Pongracic et al. (2010)</td>
<td>28.3 Lpm 2 min</td>
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<td>Chow et al. (2003); Jovanovic et al. (2004); Herbarth et al. (2003)</td>
<td>28.3 Lpm 3 min</td>
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<td></td>
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<td></td>
<td>Wu et al. (2000)</td>
<td>100 Lpm 0.5 min</td>
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<td></td>
<td>100–300</td>
<td>Anaujo et al. (2008)</td>
<td>De Ana et al. (2006)</td>
<td>28.3 Lpm 0.5–5 min</td>
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<td></td>
<td>100 Lpm 1 min</td>
<td>Bellanger et al. (2010)</td>
<td>Roussel et al. (2008); Reboutx et al. (2009)</td>
<td>28.3 Lpm 5–10 min</td>
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<td>100 Lpm 2.5 min</td>
<td>Alberti et al. (2001)</td>
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<td></td>
<td>&gt;300</td>
<td>Nesa et al. (2001); Fournel et al. (2010); Sautour et al. (2009); Six et al. (2007)</td>
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<td>100 Lpm 1–10 min</td>
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<td>&lt;100</td>
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<td>1 Lpm 15 min</td>
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<td>100–300</td>
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<td>12.5 Lpm 15 min</td>
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<td>12.5 Lpm 30 min</td>
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<td>Centrifugal sampler</td>
<td>&gt;300</td>
<td>1.2 Lpm 1.5 h P</td>
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<td></td>
<td>&gt;300</td>
<td>300 Lpm 5 min</td>
<td>Brasel et al. (2005b)</td>
<td>300 Lpm 30 min</td>
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<td>Méheust et al. (2012b)</td>
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<td>Filter collector</td>
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<td>&lt;100</td>
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<td>1 Lpm 15 min C</td>
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<td>100–300</td>
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<td>2 Lpm 30 min GF</td>
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<td></td>
<td>&gt;300</td>
<td>3 Lpm 33 min T</td>
<td>Bellanger et al. (2009)</td>
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<tr>
<td></td>
<td>3 Lpm 3 h P</td>
<td>Morrison et al. (2004)</td>
<td>Dales et al. (2010)</td>
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Lpm, liters per minute; C, cellulose membrane; G, gelatine membrane; GF, glass fiber filter; MCE, mixed cellulose ester filter; P, polycarbonate filter; T, teflon filter.
describe the particulate matter that collects on horizontal surfaces, primarily floors. Over a defined time period, suction devices or vacuum cleaners can be used to collect dust from a given area of carpets or hard floors in homes (usually 1–2 m$^2$). This approach is also useful following moisture damage and/or health complaints in workplace investigations. Furthermore, analysis of dust in ventilation ducts could be a practical indicator of the air treatment efficiency in hospitals. One of the main advantages of dust sample is that this matrix may be analyzed by different techniques (Table 2). However, variables such as the type of carpet, vacuum cleaner capture velocity and relative humidity can affect how well dust is removed from the floor (Macher, 2001). Besides, the respirable fraction of the dust and the length of the dust accumulation are not known. Passive airborne dust collection methods, such as electrostatic dust fall collectors, may thus be a low-cost means of assessing long-term fungal exposure in standardizing the time and the surface of dust accumulation (Frankel et al., 2012; Madsen et al., 2012; Normand et al., 2009; Noss et al., 2008).

Sampling strategies vary according to the indoor environment. Surface and air sampling are generally used in hospitals to confirm that the care environment is safe, especially in Europe. They are also useful in houses to quantify the exposure and evaluate the biodiversity. Dust is more often sampled in homes because it is an indicator of the past fungal exposure. Although techniques differ slightly depending on the level of fungal contamination, air sampling is the most commonly used technique in indoor environments, as it provides a better characterization of the airway exposure.

**Analytical methods**

Depending on the objective of the investigation, environmental samples are analyzed to detect either fungal particles or specific fungal compounds (Figure 1). On the one hand, spores and/or fungal fragments may be detected to assess fungal exposure with a global approach. On the other hand,
cell components and metabolites of molds known to be associated with adverse health effects may be specifically quantified to characterize the fungal risk.

Microscopic analytical methods

Microscopic evaluation of tape-lift samples may provide indications as to whether the mold growth in a wall is fresh or desiccated and historic. Microscopy is also performed to identify and enumerate airborne fungal spores collected by spore-trapping samplers (Godish & Godish, 2007). This technique is frequently used for outdoor air quality surveys and also has been used in a few indoor air studies (Adhikari et al., 2004a; Fabian et al., 2005; Fairs et al., 2010). Both dead and living microorganisms are quantified by this method (Dowues, 2005). However, identification of the fungal spores is often difficult: only a small number of fungal spore types can be identified with confidence at generic level, and significant genera such as Aspergillus and Penicillium cannot be differentiated. This method also has the disadvantages that the procedures are laborious and complicated, and the cost per sample is high.

Culture-based methods

Culture-based methods can provide quantitative and qualitative data on viable and culturable fungi from nearly all types of sample. Results are nevertheless influenced by many factors, particularly incubation conditions (such as culture medium, temperature) and competition between species. Malt extract agar (MEA) has a high sugar content and water activity favoring fast-growing species, whereas Dichloran Glycerol (DG18) allows more diversity but does not support growth of some fungi because of its low water activity. Dichloran extract agar (MEA) has a high sugar content and water activity favoring fast-growing species, whereas Dichloran Glycerol (DG18) allows more diversity but does not support growth of some fungi because of its low water activity. Both dead and living microorganisms are quantified by this method (Dowues, 2005). However, identification of the fungal spores is often difficult: only a small number of fungal spore types can be identified with confidence at generic level, and significant genera such as Aspergillus and Penicillium cannot be differentiated. This method also has the disadvantages that the procedures are laborious and complicated, and the cost per sample is high.

PCR-based methods

The use of molecular techniques to study microorganisms in the environment has increased significantly with polymerase chain reaction (PCR) amplification and DNA sequence analysis to detect and identify microorganisms (Peccia & Hernandez, 2006). Quantitative polymerase chain reaction (qPCR) is a fast method for specific identification and quantification of viable and non-viable fungal agents, and is being used more frequently owing to its low detection limit and high accuracy. Contamination of samples during analysis can nevertheless be challenging, because of the method’s high sensitivity. Its major advantage is that it can provide quantitative information quickly over a wide range of numbers ($10^{-10}$), from species level to a general group of microorganisms (Goebes et al., 2007; Stetzenbach et al., 2004).

A DNA-based mold analysis method, called Mold Specific Quantitative PCR (MSQPCR), was created for about 130 molds (Vesper, 2011). A national dust sampling and analysis campaign using MSQPCR in US homes produced a scale for comparing the mold burden in homes, called the Environmental Relative Moldiness Index (ERMI) (Vesper et al., 2007b), which was useful for the characterization of homes of severely asthmatic children (Vesper et al., 2008b). It was shown that early exposure to molds as measured by ERMI at 1 year of age significantly increased the risk for asthma at 7 years of age (Reponen et al., 2011). Fungal primers and probes sequences designed by scientists of the US Environmental Protection Agency have been used in many studies in hospitals (Bellanger et al., 2010; Morrison et al., 2004; Neely et al., 2004; Pitkäranta et al., 2008), in homes (Bellanger et al., 2009; Fairs et al., 2013; Lignell et al., 2008; Méheust et al., 2012a, b; Meklin et al., 2007), in shopping centers (Yap et al., 2009) and in day care centers (Cai et al., 2009). Other sequences were developed and used in agricultural environments (Zeng et al., 2004, 2006), and more recently in hotel rooms (Norbäck & Cai, 2011).

Cytometry methods

Few studies have applied cytometry methods for the detection of fungi in indoor environments. A flow cytometry technique was tested to detect fungal propagules labeled by fluorescence in situ hybridization (Prigione et al., 2004). In this study, propidium iodide was used as fluorescent intercalating agent to stain DNA. As this molecule is not specific for fungi, multiple parameters were used to differentiate fungal propagules from different cells and biotic debris in field samples. Solid-phase cytometry (SPC) may also be useful to quantify fungal spores in environmental samples (Méheust et al., 2013; Vanhee et al., 2009a). By combining flow cytometry and epifluorescence microscopy principles, this technique yields results within a few hours of sampling and has a highly dynamic detection range. New approaches in SPC based on immunofluorescent labeling may allow a detection of specific fungi. For example, Vanhee et al. (2009b) developed a protocol using monoclonal mouse anti-Aspergillus antibodies for the quantification of Aspergillus fumigatus in air samples.

Chemical and immunoassay methods

Cell wall components

Chemical methods for measuring total fungal exposure have been developed in order to automate fungal analysis. Ergosterol measurements are used in many studies as an index of fungal biomass, as this is the major sterol in cell membranes of hyphae and spores. However, it gives no information on the species present (Portnoy et al., 2004). Ergosterol is generally analyzed by liquid-phase chromatography (Robine et al., 2005) or by high-resolution gas chromatography/mass spectrometry (Dales et al., 2010; Park et al., 2007; Pitkäranta et al., 2008). \(1-3\)-ß-D-Glucan is a
Table 3. Culture-based methods for fungal detection in indoor environments.

<table>
<thead>
<tr>
<th>Media</th>
<th>Hospital</th>
<th>Home</th>
<th>Workplace</th>
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<tr>
<td>Sabouraud</td>
<td>27°C chl. 30°C chl. Faure et al. (2002); Fournel et al. (2010); Sautour et al. (2009); Sixt et al. (2007)</td>
<td></td>
<td>Faure et al. (2002); Fournel et al. (2010); Sautour et al. (2009); Sixt et al. (2007)</td>
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MEA, Malt extract agar; DG18, dichloran glycerol agar; RT, room temperature; n.d., temperature not determined; chl., chloramphenicol; gt., gentamicin.
biologically active constituent of the fungal wall that has been shown in several studies to be related to respiratory health disorders (Douwes, 2005). The *Limulus* amebocyte lysate (LAL) test for detecting glucans is an extremely sensitive method and quantities as small as a few picograms (a few tens of fungal spores) can be detected (Bex & Squinazi, 2006; Crawford et al., 2009; Foto et al., 2005). The glucan content has been shown to vary according to the fungal species and to be proportional to the spore surface area (Foto et al., 2004; Seo et al., 2007). Glucans are not specific to molds and are also present in vegetable cells (including pollen) and some bacteria (Chen & Hildemann, 2009). Several epidemiological studies have reported that glucans have strong immunomodulating effects. Thus, the exposure to glucans is often assessed in homes (Codispoti et al., 2010; Dales et al., 2010) and workplaces (Adhikari et al., 2011; Hansen et al., 2012).

**Microbial volatile organic compounds**

Microbial volatile organic compounds (MVOCs) produced by fungi growing on and degrading substrates may be responsible for nonspecific symptoms, such as headaches, eye, nose and throat irritations and fatigue (Wa, 2006). Microbial volatile organic compounds (MVOCs) produced by molds and by the lack of specificity of most fungal markers have often been hampered by the low levels of emission from molds and by the lack of specificity of most of the compounds emitted (Moularat et al., 2008; Polizzi et al., 2009; Schuchard & Kruse, 2009). It has been suggested that MVOC analysis could rely on the detection of several compounds forming characteristic patterns (Claeson et al., 2007; Moularat et al., 2008). An index of fungal contamination based on MVOC measurements was recently used to evaluate the exposure of French population to molds at homes (Hulin et al., 2013).

**Mycoxotins**

Mycoxotins are secondary fungal metabolites that cause a toxic response at low doses in vertebrates (Jarvis & Miller, 2005; Reboux et al., 2006). Most are non-volatile with a molecular weight below 1500 Da (Nielsen, 2003). In agricultural settings, mycoxoticosis in both farm animals and humans can result from oral or dermal exposure or inhalation of mycoxotin-contaminated grain or dust. Five major mycoxotins are found in agricultural environments: aflatoxins, ochratoxin A, fumonisins, deoxynivalenol and zearalenone (Jarvis & Miller, 2005; Lanier et al., 2010; Richard et al., 2007). Fungal isolates from water-damaged buildings may also produce highly cytotoxic trichotheccenes and sterigmatocystin, which may induce health problems upon inhalation (Polizzi et al., 2009). However, in vitro experiments showed that metabolite production is influenced by medium composition, temperature and water activity, indicating that molds are likely to generate different metabolites when they grow on building materials (Nielsen, 2003). In the last decade, public concern has increased over the potential health risks in indoor environments associated with the *Stachybotrys chartarum* species whose mycoxotins can inhibit protein synthesis and induce hemorrhaging disorders (Kuhn & Ghannoum, 2003). Identification and measurement of mycoxotins require advanced analytic instrumentation, such as gas chromatography/mass spectroscopy and liquid chromatography/mass spectroscopy (Bloom et al., 2009; Polizzi et al., 2009; Tuomi et al., 2000). Competitive enzyme-linked immunosorbent assay (ELISA) tests and array biosensors have also been developed (Brasel et al., 2005b; Charpin-Kadouch et al., 2006).

**Allergens**

Application of immunoassays in ecological and human exposure monitoring for indoor allergens is increasing. In 2000, a double immunostaining technique called halogen immunoassay (HIA) was developed to immunolabel the “halo” of allergen around particles using either allergen-specific monoclonal antibodies or human immunoglobulin E (Tovey et al., 2000). HIA may have clinical applications in quantifying personal exposure as well as identifying allergens (Green et al., 2009). Many key issues including sources of assay variability and the loss of assay specificity due to immunological cross-reactivity among fungi need to be resolved before immunoassays can be used on a routine basis for exposure and health assessment studies (Trout et al., 2004). Their current application for monitoring purposes of fungi is partly limited because of the problems associated with antibody specificity. Even phylogenetically distant species such as *Cladosporium* and *Stachybotrys* or *Wallemia* were found to share multiple epitopes with *Aspergillus* and *Penicillium* species (Schmechel et al., 2005). Some authors therefore recommend that accurate and quantitative monitoring assays for fungi should be developed with species-specific monoclonal antibodies (Schmechel et al., 2003). Quantitative ELISA results for fungal aerosols were moreover found to be influenced by differential sample processing, and thus method standardization is essential to maintain the comparability of immunometric monitoring results (Schmechel et al., 2003).

Although culture-based methods are the traditional approach in mycology, more recent methods such as chemical, immunological and PCR-based methods are frequently used in indoor environmental studies (Figure 1). All these techniques have specific advantages and limits, so there is no “gold standard method” for assessing fungal contamination. Several authors suggest that combined sampling and analysis methods should probably be used to produce a more comprehensive picture of indoor fungal flora (Niemeier et al., 2006; Pitkärinta et al., 2008; Reboux et al., 2009). We nevertheless consider qPCR assays as robust tools that should be shared by many teams in the future to assess fungal exposure in indoor environments.

**Technical challenges and outlook**

**Mold viability: Still an essential information?**

In certain environments, such as in high risk units of hospitals, the viability of a microorganism is a critical
factor since it determines infectivity. Fungal spores can remain viable for a short period or for many years, depending on the fungal species, type of spore and storage conditions. However, no common analysis method can be considered perfectly accurate for detecting only live cells. With culture-based methods, there is the well-described phenomenon of viable but non-culturable cells. Although qPCR provides a faster, more sensitive method than culture-based techniques for testing environmental samples, it does not differentiate between viable and non-viable cells without a pre-treatment step. Samples may indeed be treated with propidium monoazide, which is compatible with qPCR techniques, to discriminate between live and dead fungal cells (Vesper et al., 2008a). A cytometry method was also developed to label fungi with a viability substrate (Méheust et al., 2013; De Vos & Nelis, 2006). A precise identification of a specific fungus detected by this technique is nevertheless not possible without complementary methods. These methods can help determine the ratio of dead-to-viable fungi and contribute to the assessment of the fungal infections risk and to setting up preventive measures in hospital environments. However, viability is not a necessary requirement for the development of noninfectious diseases, since allergens and toxins are also present in dead cells.

**Fungal fragments: An underestimated reservoir of allergens?**

Several field studies have shown that the concentration of airborne fungal spores in mold problem buildings is not necessarily higher than in healthy buildings (Chew et al., 2003). This suggests that spores may not be the only agents contributing to adverse health effects in damp indoor environments. Large quantities of smaller-sized fungal fragments (<1 μm) are released together with spores from contaminated surfaces (Gorny et al., 2002; Kanaani et al., 2008). These fragments, which are probably pieces of spores and fruiting bodies, can be easily transported by air currents and more efficiently deposited in the lower airways than intact spores (Cho et al., 2005; Reponen et al., 2007). Their clinical significance is not really known. However, exposure to fine particles in ambient air has been associated with several adverse health outcomes, including respiratory and cardiac symptoms. The high number of released fungal fragments in combination with their potential to deliver harmful antigens and mycotoxins to the alveolar region of the lung, especially for young children, suggests that exposure assessments need to take account of fungal fragments (Cho et al., 2005; Gorny, 2004; McGinnis, 2004). For example, airborne *Stachybotrys chartarum* trichothecene mycotoxins were detected in fungal fragments, separated from conidia by means of filters with decreasing pore size (Brasel et al., 2005a). There is increased interest in the role of aerosolized fungal fragments following reports that the combination of hyphal fragments and spore counts improved the association with asthma severity (Green et al., 2006). Seo et al. (2007) suggested a new methodology to separate and analyze fungal fragment samples. But technological developments should be continued to provide realistic and comprehensive exposure profiles in homes and workplaces, because traditional bioaerosol sampling and analysis methods cannot detect these fine and ultrafine particles.

**Sequencing technologies: The next revolution in fungal risk assessment?**

Unlike many chemical agents, there are no thresholds for acceptable levels of fungal agents in indoor environments. More research is needed to understand the complex role fungi play in human diseases. As people are exposed to multiple fungal agents (Figure 1), it is still difficult to globally apprehend the fungal risk. The traditional culture-based methods greatly limit our view of the diversity and quantity of microbial material in environmental samples. Although PCR-based methods have been increasingly used during the last decade, they only detect specific fungi known to be present in indoor environments. The recent introduction of DNA sequencing by synthesis technology promises thus to revolutionize the global understanding of aerosol science (Peccia et al., 2011). The automated Sanger method is considered as a “first-generation” technology, and newer methods are referred to as next-generation sequencing (Metzker, 2010). The inexpensive production of large volumes of sequence data is the primary advantage of high-throughput technologies. These new technologies can be expected to have a tremendous effect on fungal biodiversity and ecology research. Only about 5% of the estimated 1.5 million species of extant fungi have been described, and sequence data are available for about 1% of the hypothesized number of fungal species (Begerow et al., 2010). At present, the large amount of sequence data obtained with high-throughput sequencing techniques contrasts with the lack of high-quality reference sequences with sufficient taxonomic information. Identification procedures of fungi need indeed to be adapted to the emerging demands of modern large-scale ecological studies. It was also shown that the alternative primers, DNA extraction methods and PCR replicates strongly influenced the richness and community composition as recovered by pyrosequencing (Tedersoo et al., 2010). Despite these technical challenges, sequencing technologies have potentials for characterizing fungal risk in indoor environments. In medical context, an accounting of pathogen exposure can be made that includes the multiple agents that are present, rather than single agents selected prior to sample analysis (Peccia et al., 2011). In home or workplace studies, similarities and differences between populations from different environments can be determined. Another sequencing strategy includes the production of a “metatranscriptome” – a list of transcribed genes, in a particular sample. Some important applications include gaining insights into how organisms regulate pathogenicity/toxin genes (Peccia et al., 2011). In the near future, these new high speed sequencing technologies should thus enable our understanding of the indoor environments and of the fungal effects on human health.

**Conclusions**

Exposure to fungal agents is more than ever a public health issue in various indoor environments (hospitals, homes, workplaces). Despite most of the usual techniques partially...
describe the fungal flora, some correlations have been identified between environmental fungal exposure and common diseases. Species-specific information is needed because fungal species may induce different health effects. The quantification of fungal compounds (glucans, mycotoxins, etc.) appears to be insufficient to assess the health risk associated with molds. So, quantitative PCR could be considered as a benchmark method for fungal exposure assessment. Increasing the throughput of analysis and improving innovative approaches, such as sequencing and mass spectrometry technologies, are important future directions in this field. However, only an interdisciplinary approach will improve research with methods that can be readily transferred from one discipline to another.

Public-health, aerosol and environmental science should be brought together into the study of indoor fungal contamination to ensure that the strategy and tools used meet the challenge.

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