Houses with mould problems
Comparison of two methods for verifying mould growth in buildings

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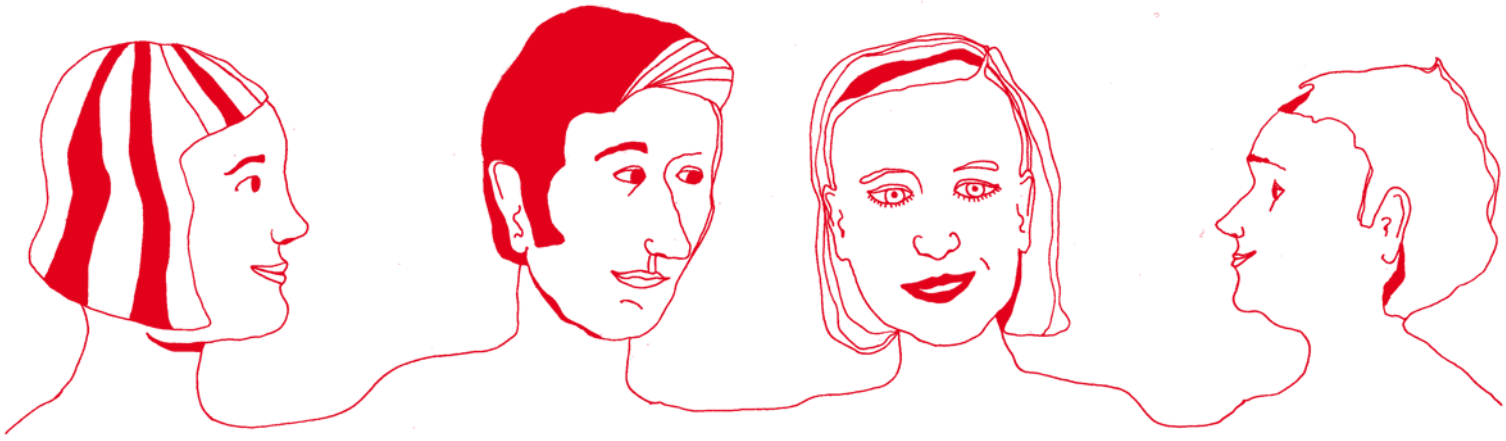

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Abstract

During the last ten years, the adverse health effects in upper respiratory system and irritation symptoms have become increasingly evident in residents living in moisture damaged houses with dampness and mould growth. In the mid of 1995, Finnish environmental health authorities faced the problem with an increasing demand for investigations to verify the microbial growth in suspected buildings and thereby taking appropriate control measures to protect the health of occupants. As an extension of an earlier relatively onerous method for quantification of microbial growth on surfaces, the Food Laboratory in Porvoo developed an applied direct streak method (DSM) aiming at a facilitation of the method verifying the presence of mould growth. The method was established in close collaboration with health inspectors. The DSM was compared to the original culture method (CM) and repeatability and reproducibility were good or very good. The DSM is a semi-quantitative method and enables the health inspectors to take surface samples without breaking the building structures and without being restricted to stiff surfaces. The results can demonstrate that the material is damaged by active viable mould growth. The DSM provides a powerful and useful tool for health inspectors to verify mould growth in buildings and it supports them to make decisions that are needed to protect the health of occupants. The cost of DSM is about one fourth of that of the more elaborate CM. The DSM is being increasingly used in Finland.

Key words

indoor air, mould growth, laboratory method, mycoecology

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CONTENTS

ABBREVIATIONS

a_w	water activity
CFU	colony forming units
CI	confidence interval
CM	culture method
DSM	direct streak method
FFP	fire and freeze protection
κ_w	weighted kappa
MEA	malt extract agar
MVOC	microbial volatile organic compounds
OTDS	organic toxic dust syndrome
TYG	tryptone yeast glucose agar

INTRODUCTION

Since 1980s, unspecific complaints associated with sick building syndrome (SBS) have been reported with increasing frequency in occupational medicine (Finnigan et al. 1984, Redlich et al. 1997, Cooley et al. 1998). SBS refers to non-specific complaints, including upper-respiratory irritative symptoms, headaches, fatigue, and rash, which are usually associated with a particular building (Redlich et al. 1997). Redlich and co-authors also proposed that SBS must be distinguished from rhinitis, asthma and hypersensitivity pneumonitis, which may be due to mould growth in buildings. However, in buildings with moisture damage and microbial growth, the exposure is always a complex mixture of particles and volatile chemicals. It is impossible to measure all possible components and to distinguish between the effects of various exposures (Husman 2000). Cooley et al. (1998) showed that certain fungi, *Penicillium* species and *Stachybotrys* species, were strongly associated with SBS. In general, indoor exposure to noxious chemical, physical, and biological hazards occur at low levels in all buildings. SBS is, therefore, rarely attributed to a single specific exposure alone. Poor ventilation has been the major cause for accumulation of indoor air pollutants and the repair of ventilation capacity has often removed the problems in SBS (Cooley et al. 1998).

During the last ten years, the adverse health effects in upper respiratory system and irritation symptoms have become increasingly evident in the residents living in moisture damaged houses with dampness and mould growth (Koskinen et al. 1999, Haverinen et al. 2001, Dales et al. 1997, Peat et al. 1998). In Finland, a prevalence study of randomly sampled houses showed that visible moisture problems were observed in 52 % and visible mould problems in 27 % of the houses (Koskinen et al. 1999). The risk of respiratory infection was significantly increased with exposure to moisture and mould. Another Finnish study among randomly sampled dwellings showed a dose-dependent trend for rhinitis, hoarseness, eye irritation, and eczema in both hand and face areas according to three-scaled severity of moisture-damaged dwellings (Haverinen et al. 2001). Several studies have shown an increased risk for sinusitis, coughing without phlegm and nocturnal coughing in moisture damaged dwellings (Haverinen et al. 2001, Koskinen et al. 1999). The health effects associated with moisture damaged dwellings can be divided in five categories: 1) irritative symptoms, 2) respiratory symptoms, 3) allergic diseases, 4) pulmonary diseases, such as alveolitis, organic dust toxic syndrome (ODTS), and 5) chronic bronchitis (Husman 2000). In a population-based case-control study in Finland, the researchers found that the risk of asthma was related to the pre-sence of visible mould and/or mould odour in the workplace (Jaakkola et al. 2002). In their study, the risk of asthma was related to none of the exposure indicators in the home.

Good evidence exist that moisture damage and possible subsequent microbial growth causes adverse health effects. An intervention study in Finland showed that mould remediation of one moisture damaged school had positive effects on teachers' health (Patovirta 2005). The number of moisture-damaged buildings is relatively high in Finland resulting in large scale health problems in the population (Nevalainen et al. 1998). In the mid 1995, environmental health authorities faced the problem with increasing demand of investigations to verify the microbial growth in suspected

buildings and thereby taking appropriate control measures to protect the health of people.

BACKGROUND

In any studies of fungal growth in buildings a working knowledge of mould ecology is needed. Various species of moulds differ in their responses to environmental conditions, in their abilities to attack various types of material, in their ability to tolerate chemical substances, and in their ability to compete with other microbes (Onions et al. 1981, Dix & Webster 1995). Furthermore, variations in external conditions may not only affect the rate of growth but can induce differences in type of growth resulting in diversity within species (Onions et al. 1981). Basic understanding of mould ecology is essential in understanding the development of microbial growth in moisture damaged buildings.

All fungi require oxygen for growth. Fungi can grow very slowly in fluid but even this is thought to depend on the oxygen content in the fluid (Onions et al. 1981). Another essential component for growth is water. Water activity, a_w, is often considered as a measure of "free" water available for microbes (Onions et al. 1981). A material with water activity a_w of 0.80, for example, is in equilibrium with air of relative humidity 80 % (Onions et al.1981). Most fungi grow only above 95 % relative humidity but many species can grow at much lower humidity. The limiting level of a_w for growth of fungi is generally considered to be about 0.65 (Dix & Webster 1995). The fungi able to grow at low level of water activity are called xerophilic or xerotolerant fungi. They can form fungal communities on dry substrata. The genus *Aspergillus* is collectively the most xerotolerant group of fungi and includes many xerotolerant species (Dix & Webster 1995). It is important to distinguish between relative humidity in the air and moisture content in the material that may differ remarkably. The humidity at a surface may be much higher or lower than measured relative air humidity a few centimetres away (Onions et al. 1981). This reflects the situation where the material is either in the process of drying or absorbing water.

Temperature is one of the cardinal factors affecting fungal growth. At high temperatures, thermotolerant and thermophilic fungi dominate. They can grow well at 45°C - 60°C. For example, *Aspergillus fumigatus* grows well at 50° (Onions et al 1981). The majority of fungi are mesophiles and grow in the temperature range 5°C - 35°C (Dix & Webster 1995). Mesophilic fungi grow rapidly on moist building materials (Pasanen et al. 1992). However, many moulds can grow well at or just below 0°C (Onions et al. 1981). Increasing temperature increases the a_w-value even if the moisture content remains constant (Onions et al. 1981).

The development of microbial growth in materials has varying dynamics over time. In favourable conditions, the primary colonizing mould species compete with each other vigorously and grow rapidly using up all nutrients available on the material. Interactions between the microbes and with the changing nutritional environment cause continual changes in microbial growth. The pioneer species will be replaced with other, secondary and later tertiary species, which are able to degrade different compounds, e.g. cellulose, in materials. These time-related changes in fungal community structures are the so-

called fungal successions (Dix & Webster 1995). At the early stage of colonization, the fungal communities are typically composed of large number of different species (Dix & Webster 1995). As the community matures, only one or two obvious dominants grow on the substratum. The interactions between the fungal species range from stimulation of growth, through mutual tolerance and degrees of intolerance, to the development of highly aggressive forms of competitive behaviour. Many fungi produce fungistatic or fungicidal metabolites (antibiotics/mycotoxins) which diffuse from hyphae and/or evaporate into the air and slow or stop the growth of competitors (Dix & Webster 1995). Mycotoxins/antibiotics are also bound to spores. Some fungi are parasites to other fungal species (mycoparasitism). For example, *Trichoderma* species express mycoparasitic behaviour and in addition, produce mixtures of powerful volatile and non-volatile mycotoxins that are toxic to other fungus species (Dix & Webster 1995).

Microbial growth on building materials consists of moulds, yeasts and various bacteria, e.g. actinobacteria. Actinobacteria are bacteria which can form aerial mycelium. They, in turn, can destroy fungal spores (Dix & Webster 1995). Actinobacteria produces volatile organic compounds which affect destructively on living cells (Jussila et al. 2001). The microbial growth produces spores, various kinds of mycotoxins, bacterial endotoxins (gram negative cell wall structures), and microbial volatile organic compounds (MVOC) that all may have adverse effects to human health (Husman 2000).

Usually, the initial stage of interior microbial growth begins with water leaks that wet various building materials. If these wetted materials are not properly dried up, fungal growth may occur (Cooley 1998). Consequently, the microbial growth occurs usually behind the interior layer of buildings. The growth of microbes on building materials does not necessarily lead to detectable mould or spore concentrations in the indoor air and therefore, surface and material samples are preferred as measures of exposure (Husman 2000). Spore release in the air depends on the type of fungi and their respective growth habits under the given conditions. The fraction of spores released from the surface is different for different fungi (Kildesø et al. 2000).

The studies on association between adverse health effects and microbial growth in buildings have often the difficulty to define exposure in a valid way. In most studies, the exposure has been defined by visual inspection for moisture damage (Koskinen et al. 1999, Haverinen et al. 2001, Jaakkola et al. 2002). On visually moisture damaged materials, a heavy microbial growth may be detected by surface sampling whereas air sampling may show no elevated levels of microbes in the air (Meklin et al. 1996). Classification of moisture damaged buildings based on visual inspection of moisture or mould growth includes a potential to methodological bias lowering the power to detect possible associations between the symptoms and mould growth in buildings. As mould growth may occur behind the interior surface of the dwellings it is not necessarily detected by visual inspection only. Furthermore, if the surface sampling is restricted to visible interior layers only, which usually is the case in inspection visits, the microbial growth may not be detected. In studies of school buildings, *Stachybotrys* was not found in any of the air samples but was isolated only with swab samples from materials with visible mould growth (Meklin et al. 1996, Cooley et al. 1998). In 11 schools out of 48 the swab samples were the only indicators of possible fungal growth in the interior

(Cooley et al. 1998). The problem in measuring exposure has been the lack of accurate methods to measure the microbial growth in the building materials.
The current traditional culture methods underestimate the microbial exposure both in quantity and in quality (Nevalainen et al. 2005).

As the exposure to mould spores and mould growth related compounds may occur both at work and at home the environmental health officers and occupational health officers need an unambiguous tool to verify whether there is a mould growth in building material or not and whether it is significant or not. If possible, the suspected material should be examined *in situ* and notes and photographs taken before and after sampling (Onions et al. 1981). This is often difficult or impossible because there may not be visible signs of moisture damage and other measurements may show elevated moisture levels *behind* or *under* the interior surfaces. In any buildings, it is not easy to assess outer building structures behind or under the interior surfaces. Therefore, it is very important to be able to verify mould growth in buildings without breaking down structures.

The Finnish Ministry of Social Affairs and Health has released instructions for indoor air quality assessment including detailed methods for microbial investigations (Sisäilmaohje 1997). The microbial methods consist of indoor air sampling and surface sampling. Surface sampling occurs with a template of 10 x 10 cm using sterile swabs and specified dilution medium. However, the recommended method restricts the sampling on flat surfaces as template should be used. This in turn results in strong underestimation of possible microbial growth in buildings. The Food and Environmental Laboratory in Porvoo developed an applied direct streak method (DSM) to verify microbial growth in moisture –damaged buildings in close collaboration with health inspectors. The method enables the health inspectors to take surface samples without breaking the building structures unnecessarily and without being restricted to a stiff template. After standardization of the method, the results of the DSM were compared to the results of the standard culture method (CM) suggested by the Finnish Ministry of Social Affairs and Health.

AIM OF THE STUDY

The aim of the study was to assess the usefulness of a simplified method for verifying mould growth in buildings by comparing it to the existing traditional method.

MATERIALS AND METHODS

A cross-sectional two-centre study was conducted in buildings notified to the municipal environmental health authorities in Porvoo and Lahti cities between January and April 1999. The buildings under investigation were any building associated with a suspicion of moisture damage by the users or occupants and followed by the preliminary interviews of the occupants suggesting probable moisture damage in the

buildings. These buildings (n=15 in Porvoo and n=7 in Lahti) comprised of private houses and public buildings, e.g. schools.

Health inspectors performed all interviews according to their daily routines. They visited the suspected houses and buildings to make interviews, visual inspections and routine measurements, and to take samples for microbiological investigation from suspected places. One comparison sample per object was taken before the sampling from suspected damaged materials.

Direct streak method (DSM)

The sampling area was a visually estimated area of 3 x 3 cm. In addition, corners and chinks were as good sampling areas as visible flat surfaces. The sampling was performed with sterile cotton buds using sterile water to slightly moisture the bud before swabbing. The area was swabbed by circling the cotton bud three times in a cross-sectional way in order to get an even distribution of loose surface material around the bud. In case of sampling from corners and chinks, the swab was rolled properly within the chink to get an even distribution of loose material around the bud. The swab was cultured directly on to the agar plates by streaking in an angle of 20-30º, five lines per plate, taking care of not touching the inner edges of the plates. The bud was turned about 90º between the cultivation of each plate. The cultivation order was first on MEA and then on TYG to avoid possible inhibitory effect from the TYG plate.

A semi-quantitative 5-scale was used to read and report the results (Table 1).

Table 1 The scale for the microbial growth assessment with the direct streak method (DSM)

Semi-quantitative result		Approximate corresponding cfu/plate[1]
No growth	-	0
Light growth	+	~ 1-10
Moderate growth	++	~ 11-50
Heavy growth	+++	~ >50, countable colonies
Very heavy growth	++++	colonies not countable

[1]cfu = colony forming unit

The reading of total growth of yeasts and moulds was based on visual assessment and the use of a stereo microscope. The identification of dominant fungal species was made by stereo microscope and/or phase contrast microscope.

The fungal growth of three or four plus suggested the formation of fungal communities on the surface, i.e. suggesting a mould growth on the material. In case of two plus, the identification of fungal species was crucial. The growth of many different primary species was a sign of early fungal competition and merely implied a dusty environment.

With the two plus, the growth of indicator species may give a hint of a mould growth in building material near the sampling site and point to a possible moisture damaged area. The fungal growth of one plus was not significant. However, a single colony of *Stachybotrys* was considered as a significant finding.

Culture method (CM)

At the laboratory, the diluted sample was analysed according to the general laboratory procedures for quantitative microbial analysis based on the national instructions by the Ministry of Social Affairs and Health (Sisäilmaohje 1997).

The quantitative results were obtained according to the national instructions and were reported as colony forming units, cfu/cm^2 or as cfu/inspected area (in case of a chink).

Training of health inspectors

Before the start of the study, two health inspectors from Porvoo and one from Lahti were trained for sampling and cultivation with DSM using moisture-damaged building material samples in the laboratory of Porvoo. The health inspectors cultivated in duplicate ten samples resulting in 60 agar plates. The training included also the use of protective clothing, i.e. breathe shelter (FFP 3), hair cap, separate coat, and gloves at the time of sampling.

Sampling and cultivation

According to national instructions, the sampling area for the CM is 10 x 10 cm with a template but in this study the sampling area approximately 3 x 3 cm according to the DSM was used. The trained health inspectors took duplicate swab samples in a standardised way from surfaces, corners and small chinks. The swab samples were taken from a diversity of material surfaces suspected or known to harbour mould growth. The first swab was cultivated directly on the plates according to the direct streak method. The corresponding duplicate swab was diluted in 5 or 10 ml of generally used peptone salt water (NMKL 1998) including Tween 80 (Pasanen 1992) and transported refrigerated to the food and environmental laboratories in Porvoo and Lahti respectively. In the laboratories, the duplicate sample was cultivated according to the traditional culture method.

The analysed microbial groups were, total growth of yeasts and moulds, identified dominant fungal species, and actinobacteria. Malt extract agar (MEA) for yeasts and moulds and tryptone yeast glucose agar (TYG) for actinobacteria were chosen for the study based on the national instructions (Sisäilmaohje 1997). The plates with diameter of nine cm were used. The agar plates and the dilution liquids were prepared in the laboratories from commercially available dry media (Labema®) and the plates were quality-checked before use.

The agar plates were put in loosely sealed plastic bags and incubated at 23 ± 3ºC for up to seven days for yeast and moulds and 14 days for actinobacteria.

Harmonising the reading of DSM plates

The laboratory personnel responsible for reading the DSM plates were trained for reading the agar plates. The agar plates that were used for harmonising the readings were the same that were obtained from training of the health inspectors. The incubated plates were coded in random order before reading to avoid memory bias. All detected deviations were discussed and agreed after readings. The same trained laboratory persons in Porvoo and in Lahti read all the DSM plates during the study.

Cut-off values

According to the national instructions, verified microbial growth in building materials may constitute a health hazard for occupants. The verification should be based on comparison of the microbial growth between the suspected sample and the comparison sample. The comparison sample should always be taken first and it should be taken from the same type of but not-damaged material as the suspected sample. For CM, the microbial growth was considered as "verified" if the difference between the comparison sample and the suspected sample was at least 100-fold for yeast and moulds and 10-fold for actinobacteria. In this study, only one comparison sample was taken from each object. The results of comparison samples showed consistently very low microbial growths (<10 cfu/m^2 for CM and maximum one plus for DSM) both in Porvoo and in Lahti. Applying the results of comparison samples, a cut-off value of 1000 cfu/m^2 for total growth of yeast and moulds, and for identified dominant fungal species was determined. Respectively, a cut-off value 100 cfu/m^2 was determined for actinobacteria.

For DSM, the interpretation was based on previous years' experience and the correspondence of results of comparison samples with CM. The microbial growth was considered as "verified" if the total fungal growth was three or four plus or actinobacteria growth was two, three, or four plus.

Agreement of interpretation

The agreement of interpretation with the two methods was assessed in three categories of results: for total growth of yeasts and moulds, for identified dominant fungal species, and for actinobacteria after interpreting the results according to the cut-off values.

Statistical analyses

For laboratory persons, the repeatability of readings was calculated by $\kappa_{w,}$ weighted kappa, using results from training and harmonising session.

The repeatability of DSM was calculated by κ_w with 95 % confidence intervals (Altman 1997) for duplicate plates. The repeatability of CM was assessed according to instructions of British Standard Institution and described by Bland & Altman (1986). For paired logarithm transformed colony counts, mean and standard deviation was calculated. A paired t-test was calculated to test if the mean difference was significantly different from zero. If the t-test gave a non-significant p-value, the repeatability was assessed based on the percent of differences outside mean ± 2 standard deviations (std). If the colony count was below the limit of detection, e.g. <10 cfu/cm^2, a logarithm value of 0.9 was used.

After three months from the beginning of the study, 11 agar plates, which had been cultivated in Porvoo from real samples, were read at first in Porvoo and the next day in Lahtis. The results were combined with the training session results for reproducibility calculation of DSM. The reproducibility of DSM was calculated by κ_w. The reproducibility of CM was calculated by comparing the differences between duplicate logarithm transformed colony counts in Porvoo and Lahti with Kruskal-Wallis-test.

The analyses were performed with Epi-info 6.04 (CDC, Atlanta, US) and Excel.

Dispersion of DSM

The validation of method has been undertaken in Porvoo laboratory. The method has been presented in separate educational sessions for local environmental health authorities, e.g. health inspectors as well as for laboratory technicians in Finland.

RESULTS

Repeatability of the reading

A total of 30 reading results were available to calculate personal repeatability in laboratories. For total fungal growth, the κ_w of readings for laboratory persons in Porvoo and Lahti were 0,95 and 0,96 respectively. For actinobacteria, only repeatability for laboratory person in Porvoo could be calculated from 30 plates. The κ_w for readings of actinobacteria in Porvoo was 0,93.

Repeatability of DSM and CM

The repeatability of DSM was very good or good in all three target microbial groups (Table 2).

Table 2. The repeatability of DSM in the microbial result groups.

Category	N	κ_w	95 % CI	Assessment
Total growth of yeasts and moulds	125	0.76	0.74-0.78	Good
Identified dominant fungal species	71	0.84	0.82-0.86	Very good
Actinobacteria	117	0.76	0.75-0.76	Good

The repeatability of CM was good for total growth of yeasts and moulds, and for actinobacteria (Table 3). For identified dominant fungal species, the repeatability was not good as 8.5 % of differences lied outside the range mean ± two standard deviations.

Table 3. The repeatability of CM in the microbial result groups.

Category	N	d_{mean} [1]	d_{mean}±2std [2]	p	% [3]
Total count of yeasts and moulds	125	0.055	-0.625-0.735	0.071	4.8
Identified dominant fungal species	71	0.034	-1.130-1.198	0.622	8.5
Actinobacteria	117	-0.019	-0.385-0.347	0.266	2.6

[1]Mean difference, log
[2]Standard deviation
[3]Percent of differences outside mean ± 2std

Reproducibility of DSM and CM

For DSM, duplicate independent readings from 60 + 11 plates were available for the calculation of reproducibility of total fungal growth. The κ_w for readings of total fungal growth was 0.93 suggesting very good agreement between the readings in two laboratories. A total of 117 + 32 identified fungal species from 60 + 11 plates were available for calculation of reproducibility of identified dominant fungal species. The weighted kappa for identified dominant fungal species was 0.73 suggesting a good agreement in the identification of fungal species on the DSM plates in both laboratories.

The duplicate results by culture method could not be shown to be different by laboratories for total count of yeasts and moulds (p=0.277) and for actinobacteria

(p=0.518). For identified dominant fungal species, the laboratories had different results (p=0.021) suggesting the high variation between the duplicate agar plates.

Diversity of fungal genera

The diversity of fungal genera was slightly larger with DSM than with CM. A total of 22 different fungal genera were identified with DSM and 18 with CM. The most commonly identified fungal genera with both methods were *Penicillium*, yeasts, and *Aspergillus* (Table 4). Four genera were detected with DSM only and one genera with CM only.

Table 4. Identified fungal genera in DSM and in CM.

Fungal genera	DSM		CM	
	N	%	N	%
Penicillium	54	32.7	27	29.0
Yeasts	25	15.2	19	20.4
Aspergillus	22	13.3	9	9.7
Cladosporium	15	9.1	7	7.5
Phoma	8	4.8	7	7.5
Chaetomium	7	4.2	3	3.2
Aureobasidium	6	3.6	2	2.2
Scopulariopsis	6	3.6	5	5.4
Ulocladium	4	2.4	2	2.2
Tritirachium	2	1.2	1	1.1
Acremonium	2	1.2	1	1.1
Mucor	2	1.2	0	0.0
Trichoderma	2	1.2	1	1.1
Paecilomyces	2	1,2	2	2,2
Sphaeropsidales	1	0.6	0	0.0
Fusarium	1	0.6	0	0.0
Rhizopus	1	0.6	0	0.0
Botrytis	1	0.6	2	2.2
Phialophora	1	0.6	2	2.2
Stachybotrys	1	0.6	1	1.1
Exophiala	1	0.6	1	1.1
Alternaria	1	0.6	0	0.0
Doratomyces	0	0.0	1	1.1
Total	165	100.0	93	100.0

Agreement of interpretation

The best agreement in DSM and CM was for total growth of yeasts and moulds (Table 5). For identified dominant fungal species, the agreement was also good and for actinobacteria moderate.

Table 5. Agreement of DSM and CM for total growth of yeasts and moulds, identified dominant fungal species, and actinobacteria.

Category	N	κ_w	95 % CI	Assessment
Total growth of yeasts and moulds	125	0.81	0.65-0.96	Very good
Identified dominant fungal species	71	0.61	0.42-0.81	Good
Actinobacteria	117	0.57	0.29-0.84	Moderate

Dispersion of DSM

The method has been validated in Porvoo laboratory for total growth of yeasts and moulds and accredited by the Finnish Accreditation Service since 2002. This seems so far to be the first and only accredited method to verify mould growth in buildings in Finland.

The method has been presented in several educational occasions for health inspectors and laboratory technicians: in Kauhajoki, Lahti, Joensuu, Hyvinkää, and Turku region. The educational occasions have included presentations of mycoecology, detailed presentation of the direct streak method, identification of fungal genera, interpretation of DSM results taking into account the indicator organisms, safety at work, and quality assurance. The method is in routine use in Porvoo and in Lahti.

In addition, the method has been presented orally at the 7th Congress of the European Confederation of Medical Mycology – ECMM in Rhodes, Greece, June 16-19, 2001 (Takkinen and Löfroth 2001).

DISCUSSION

In a routine examination for fungal growth on a particular surface with the plating method an area is selected and swabbed. The same would apply to a routine examination by the direct streak method. The comparison between the plating method and the direct streak method has, however, a practical problem in that two identical samples are required. As any selected area is not microbiologically identical to another area even if they are close to each other, the problem was solved in this study by swabbing the same area with two cotton buds successively covering narrow streaks of the selected area. This may theoretically sample only half of the area for each method. However, the microbial particles were evenly distributed on the two cotton buds representing two identical microbial samples as well as possible and being thus comparable.

It is well known that the cultivation of moulds with common plating method may show remarkable over-dispersion and thus, greater variance than expected resulting in colony counts with high uncertainties. This could be seen in this study as a poor repeatability and reproducibility of identified fungal species with the plating method. As a semi-quantitative method, the direct streak method is not too specific but allows high enough sensitivity as it smooth the variation of microbial growth on agar plates without losing the ability to distinguish the significant growth from unspecific growth.

The fact that a selected area is not identical to another also limits the study of the repeatability and reproducibility of the methods. The repeatability of the direct streak method was judged from classification of duplicate plates, i.e. whether or not they were classified in the same group. However, a good agreement with duplicate plates with DSM showed a very good repeatability. With direct streak method, the sources of variation are much more limited than with traditional plating method, which includes steps of diluting and homogenizing. The reproducibility of the direct streak method was evaluated combining the results in a harmonization study with real samples later. The samples in harmonization study represented a good mixture of different levels of microbial growth. However, they may be slightly different from samples taken *in situ*. On the other hand, the harmonization samples represented the microbial growth at the time of investigation and thereby were perfectly suitable for the study purpose. The repeatability of the plating method was evaluated by comparing the difference between duplicate plates of all samples and the reproducibility of the dilution method was assessed from the same data by ranking the differences between duplicate plates and analyzing whether or not a systematic difference was present between the two laboratories. These methods provide a suitable way to assess the method quality parameters.

The official Finnish guidelines for moulds on surfaces comprise limits above which the material is considered to be damaged. The limits are based on comparison of samples taken from non-damaged and suspected damaged surfaces. For total count of yeasts and moulds, the difference between the two samples should be 100-fold. In our study, 22 comparison samples taken from each object showed very low levels of microbial growth with both methods. It is reasonable to think that any “healthy” surface does not contain a heavy microbial load. Moreover, it is impossible to assess by visual inspection

and technical measurements that a surface is non-damaged. Therefore, it was feasible to set up the limits for total count of yeasts and moulds as 1000 cfu/cm^2 and 100 cfu/cm^2 for actinobacteria. The classification by groups in the direct streak method shows that the limit might be set at +++ for total fungi and yeast and + for actinobacteria.

The number of samples showing growth of actinobacteria was limited in our study. The comparison of the two methods for actinobacteria needs more data.

A related method for bulk material, in which the material is placed directly on the plates, has been suggested by Reiman et al. (1999). The authors compared their direct plating method with a culture method and found that the highest of both classifications agreed well and that there was a substantially high possibility of detecting damaged material by their simplified method. They also found more fungal genera with direct plating method like we did with direct streak method in our study.

Practical experience has shown that the simplified direct streak method, presented here, takes about one fourth of the personnel and material resources compared to the more laborious culture method. However, it gives reliable information about the viable microbial growth on the surfaces. The reduction of the swabbed area from 100 to nine cm^2 also enables the sampling of odd surfaces such as corners and edges. In addition, it is not prone to the amount of sources of variation, which may weaken the reliability of colony counts by culture method remarkably. The experience in Lahti and in Porvoo has shown that the health inspectors learn the sampling and streaking quickly. As with any method used to verify mould growth in buildings, a personal protection at the site of sampling is extremely important and can not be over-stressed. The method is in routine use in Lahti and in Porvoo and some health inspectors have started to use it in Joensuu and in Kauhajoki. However, a nationally approved recognition for the method and national support for method dispersion is still needed.

CONCLUSIONS

The overall conclusion is that the direct streak method is reasonably rapid and inexpensive and that it gives results comparable to those of the culture method. It provides with a powerful and useful tool for health inspectors to verify mould growth in buildings and it supports them to make decisions that are needed to protect the health of occupants.

The direct streak method is semi-quantitative and can demonstrate that the material is damaged by mould growth as is the case for the culture method. The presence of mould growth on surfaces in a building can only suggest that people living in the building might be exposed to harmful biological dust and volatile compounds and much more elaborate studies are required to find the association between mouldy buildings and the health of the occupants. For these studies, the direct streak method provides an easy and reliable method to define the exposure of occupants to the fungal elements. However, there is already strong evidence that microbial growth on building surfaces causes adverse health effects in occupants.

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