National Collaborating Centre for Environmental Health

Centre de collaboration nationale en santé environnementale

NOVEMBER 2014

NCCEH Mould Investigation Toolkit
Overview of Microbial Sampling Methods

Table of Contents

This document is one of several in the NCCEH Mould Investigation Toolkit. *Production of this document has been made possible through a financial contribution from the Public Health Agency of Canada.*

Table 1. Overview of Microbial Sample Collection Methods

Sampling Method		Purpose	Process	Advantages	Disadvantages	Results
A	**Air Sampling**					
1	**Liquid Impinger**	Collect viable and non-viable organisms and spores	Bubble air through a liquid medium	■ Suitable for collection of a variety of organisms ■ Can collect a greater diversity of organisms compared with other sample methods ■ Cannot be overloaded ■ Can identify fungi, bacteria, and yeast to the species level	■ Not effective for very small organisms ■ Uneven collection of fungal spores ■ Possible sterilization issues ■ More difficult to collect than other methods ■ Requires cooling packs for transport ■ Cannot conduct personal monitoring easily ■ Requires a minimum of seven days for incubation following sampling	CFU/m^3 CFU/ml

Sampling Method		Purpose	Process	Advantages	Disadvantages	Results
A	**Air Sampling**					
2	**Fungal Spore Sampling/Spore Traps**	Collection of fungal spores	Draw air over a sticky slide where fungal spores and other materials adhere	■ Quick turnaround time for results ■ Easy to sample ■ Identify spores which are present but no longer viable (or do not culture well) as well as viable spores ■ Method can be used to identify and quantify pollen, hyphal fragments, hair, skin cells, etc. present in the air, which can contribute to indoor air quality issues	■ Sample time is short (e.g., up to 10 minutes) ■ Can overload cassettes easily, especially if dusty or if other materials are present (pollen, mites, dander) ■ Counting errors can occur because of the presence of other particles, uneven distribution of spores, and difficult-to-see spores (transparent) ■ Cannot determine viability of spores ■ Cannot be used to identify bacteria or yeasts ■ Cannot identify specific species present ■ Many spores are morphologically similar and cannot be differentiated to the genera level (e.g., *Penicillium*, *Aspergillus*) ■ Genera with small spores may be underreported because they are not captured on the slide during sampling	Spores/m^3 Spores/cassette

Sampling Method		Purpose	Process	Advantages	Disadvantages	Results
A	**Air Sampling**					
3	**Impactor Samplers (e.g., Andersen, RCS, etc.)**	Collect viable and non-viable organisms and fungal spores	Draw air through sieves or slits of specific size and shape onto an agar petri dish or agar strip	■ Suitable for identification of a variety of organisms ■ Can identify fungi, bacteria, and yeast to the species level ■ Can assess the viability of organisms ■ Some samplers operate on batteries and do not require a power source ■ Some models can be used to collect different sized organisms (size fractions)	■ Sample time is short (e.g., 1 to 5 minutes) ■ Can overload the agar plates or strips ■ Need a power source for some samplers ■ Sampling for too long or at wrong flow rate can cause desiccation of organisms, or loss of organisms due to bounce ■ Limited agar media available for some models ■ Choice of sampling equipment and media can influence the sample results (e.g., MEA is a good broad spectrum agar for fungi, but slow growing fungi, such as *Stachybotrys*, do not grow well on it) ■ Cannot conduct personal monitoring ■ Requires the organisms or spores be alive and survive the sampling and growth process ■ Does not indicate the presence of non-viable spores, which may be capable of producing allergies or irritation ■ Requires a minimum of seven days for incubation following sampling	CFU/m^3 CFU/plate

Sampling Method		Purpose	Process	Advantages	Disadvantages	Results
A	**Air Sampling**					
4	**Sedimentation Settling Plates**	Collect viable and non-viable organisms and spores	Uncover agar plates and allow bioaerosols to settle onto plate	■ Easy to use, no power required ■ Suitable for collection of a variety of organisms ■ Can identify organisms to the species level	■ Can overload the agar plates ■ Sampling for too long can cause desiccation of organisms or agar ■ Cannot conduct personal monitoring ■ Requires the organisms or spores be alive and survive the sampling and growth process ■ Does not indicate the presence of non-viable spores, which may be capable of producing allergies or irritation ■ Requires a minimum of seven days for incubation following sampling ■ Generally thought to be less accurate or reliable compared with other methods, as method is gravity dependant and only captures organisms that happen to settle on the plate ■ Biased against small-spored genera such as *Aspergillus* and *Penicillium* that stay airborne longer ■ Subject to false negatives if room air is very still; species present do not culture well, or predominant species have poor dissemination in air	CFU/plate CFU/hour

Sampling Method		Purpose	Process	Advantages	Disadvantages	Results
A	**Air Sampling**					
5	**Airborne Particulate**	Collect viable and non-viable organisms and spores	Collection of airborne particulate onto a membrane filter using a pump or vacuum	■ Can identify fungi, bacteria, and yeast to the species level ■ Some operate on batteries and do not require a power source ■ Suitable for collection of a variety of organisms ■ Can sample for longer periods of time (e.g., hours to days) and can collect personal samples	■ Need a power source for some samplers ■ Sampling method can result in desiccation of organisms so they do not survive the sampling process ■ Does not indicate the presence of non-viable spores, which may be capable of producing allergies or irritation ■ Requires a minimum of seven days for incubation following sampling	mg/m^3 ng/m^3
B	**Bulk Sampling**					
6	**Bulk Sample of Material**	Identification of viable and non-viable organisms and spores	Collection of a bulk sample of material (e.g., drywall) for analysis	■ Suitable for identification of a variety of organisms. Can identify fungi, bacteria, and yeast to the species level if sample is cultured ■ Can analyze microscopically for fungi and bacteria to the genera level and fungal spores if analyzed microscopically ■ Quick turnaround time for microscopic analyses ■ May reveal fungal spores than have not yet become airborne ■ May reveal fungi or bacteria that are not routinely airborne	■ Sampling collection and shipment can result in desiccation of organisms so they do not survive the sampling process ■ Requires a minimum of seven days for incubation following sampling if sample is cultured ■ Do not indicate concentrations in air	CFU/g CFU/area Qualitative assessment of presence of organisms or spores.

Sampling Method		Purpose	Process	Advantages	Disadvantages	Results
B	**Bulk Sampling**					
7	**Dust**	Identification of viable and non-viable organisms and fungal spores in surface dust	Collection of dust sample (e.g., bulk sample) for analysis. Dust may be collected with the use of a vacuum and filter	■ Suitable for identification of a variety of organisms ■ Can identify fungi, bacteria, and yeast to the species level if sample is cultured ■ Can indicate if organisms presently, and historically, in the air have settled ■ Can be analyzed via direct microscopic examination and cultured ■ Microscopic analysis has a quick turnaround time	■ Sampling collection and shipment can result in desiccation of organisms so they do not survive the sampling process ■ The results can be difficult to interpret ■ Requires a minimum of seven days for incubation following sample collection if analyzing via culturable methods ■ Results do not indicate concentrations in air ■ Grab samples, usually, may not be representative	CFU/g Qualitative assessment of presence of organisms or spores
8	**Tape**	Identification of microbial organisms and fungal spores on surface of a material	Stick a piece of clear tape to the surface of a material, and then stick the tape to a microscope slide	■ Can identify fungi and yeasts to genera level and fungal spores in air via direct microscopic examination ■ Can sometimes identify hyphal fragments and conidia, which indicate active growth rather than just settled spores ■ Quick turnaround time on analyses ■ May reveal fungal spores than have not yet become airborne ■ May reveal fungi or bacteria that are not routinely airborne	■ The results can be difficult to interpret ■ Sample collection process can damage microbial organisms resulting in lab not being able to identify organisms ■ Results do not indicate concentrations in air ■ Grab samples usually, may not be representative. ■ Does not indicate viability of organisms.	Qualitative assessment of presence of spores, hyphae, and fruiting structures

Sampling Method		Purpose	Process	Advantages	Disadvantages	Results
B	**Bulk Sampling**					
9	**Swab / Wipe**	Identification of viable and non-viable organisms and fungal spores on the surface of a material	Use a sterile swab or a wipe moistened slightly with sterile water to sample the surface of a material	■ Suitable for identification of a variety of organisms ■ Can identify fungi, bacteria, and yeast to the species level if sample cultured ■ Quick turnaround time on direct microscopic analysis ■ May reveal fungi or bacteria that are not routinely airborne	■ Sampling collection and shipment can result in desiccation of organisms so they do not survive the sampling process ■ Requires a minimum of seven days for incubation following sampling if sample is cultured ■ Results do not indicate concentrations in air ■ Grab samples, usually, may not be representative ■ Direct microscopic examination may disturb and/or separate intact fungal structures during preparation of sample slides; therefore, direct examination seldom recommended	CFU/swab CFU/area
10	**Surface Contact Plate**	Identification of viable and non-viable organisms and fungal spores on the surface of a material	Press the surface of an agar plate against the surface of a material	■ Suitable for identification of a variety of organisms ■ Can identify fungi, bacteria, and yeast and fungal spores to the species level ■ May reveal fungi or bacteria that are not routinely airborne	■ Sampling collection and shipment can result in desiccation of organisms so they do not survive the sampling process ■ Requires a minimum of seven days for incubation following sampling if sample is cultured ■ Results do not indicate concentrations in air ■ Grab samples, usually, may not be representative	CFU/plate

Sampling Method		Purpose	Process	Advantages	Disadvantages	Results
C	**Water Sampling**					
11	**Bulk Water Sample**	Identification of microbial organisms in water samples	Collect sample of water into a clean sterilized bottle. If collecting chlorinated water samples, a chemical to neutralize the chlorine is added	■ Suitable for collection of a variety of organisms ■ Can identify fungi, bacteria, and yeast to the species level ■ May reveal fungi or bacteria that are not routinely airborne	■ Requires a minimum of seven days for incubation following sampling ■ Results do not indicate concentrations in air	CFU/ml
D	**Microbial By-Products**					
12	**Endotoxins in Air***	Identification of toxins associated with gram negative bacteria	Collect endotoxins onto an EndoFree polystyrene cassette using a sampling pump. Analyzed using the endotoxin-specific LAL assay	■ Method is suitable for collection of personal samples ■ There are exposure guidelines for endotoxins ■ Useful as indicator of possible gram negative bacteria contamination when growth is not visible ■ Useful in the assessment of possible health symptoms	■ Require a pump to collect samples ■ Sample results can vary between labs depending on sample cassette used, extraction, and analyses method utilized	Endotoxins Units / m^3 ng/m^3

Sampling Method		Purpose	Process	Advantages	Disadvantages	Results
D	**Microbial By-Products**					
13	**Mycotoxins in Air***	Identification of toxins produced by some fungi	Collection of mycotoxins on specialty filter cassettes using a sampling pump	■ Useful as indicator of possible fungal contamination when growth is not visible, and for assessment of possible health symptoms ■ Long-term and personal monitoring can be conducted	■ Require high levels of fungal spores to be present in air before mycotoxins can be detected with accuracy (>100,000 spores/m^3). More commonly, bulk or dust samples are analyzed ■ Absence of mycotoxins on sample results does not mean absence of fungal mass (fungi do not produce mycotoxins all the time) ■ Analysis of samples is expensive ■ Regular turnaround on samples is a minimum of five business days ■ Limited number of labs that perform mycotoxin analysis ■ List of mycotoxins analyzed for is limited ■ Analysis methods may not have been validated ■ No exposure limits or guidelines	ng/m^3

Sampling Method		Purpose	Process	Advantages	Disadvantages	Results
D	**Microbial By-Products**					
14	**Microbial Volatile Organic Compounds (MVOC) in Air**	Identification of VOC associated with microbial growth	Collection of MVOC using whole air gas canister. Alternatively, collection of MVOC on an anasorb CMS sorbent tube	■ No pump or electricity required to collect samples with a whole air gas canister ■ Useful as an indicator of possible microbial contamination when growth is not visible, assessment of odours, and assessment of possible health symptoms ■ Long-term and personal monitoring can be conducted	■ Analysis of samples is expensive ■ MVOC are not unique to microorganisms; there can be many other sources of MVOC in indoor environments. Interpretation can be difficult as a result ■ Analysis typically takes 5 to 10 business days	ppm mg/m^3
15	**β-1,3-D-Glucans in Air**	Identification of β-1,3-D-glucans associated with the cell walls of fungi	Collection of β-1,3-D-glucans on a polycarbonate filter cassette using a sampling pump. Analyzed using the glucan-specific LAL assay	■ Useful as indicator of possible fungal contamination when growth is not visible, and assessment of possible symptoms (headaches and respiratory tract inflammation) ■ Long-term and personal monitoring can be conducted	■ Analysis by a laboratory required ■ Limited number of labs that perform analysis of environmental samples for β-1,3-D-glucans ■ Cannot be used to identify specific fungi or fungal spores ■ Non-fungal sources can impact results (e.g., some bacteria product glucans)	ng/m^3

Sampling Method		Purpose	Process	Advantages	Disadvantages	Results
D	**Microbial By-Products**					
16	**Ergosterol in Air**	Identification of ergosterol (a unique sterol associated with fungi)	Collection of ergosterol on specialty filter cassettes using a sampling pump	■ Useful as indicator of possible fungal contamination when growth is not visible, and assessment of possible symptoms ■ Long-term monitoring can be conducted	■ Analysis by a laboratory required ■ Limited number of labs that perform analysis of environmental samples for ergosterol. This analysis is seldom used in a fungal assessment ■ Cannot be used to identify specific fungi or fungal spores ■ No exposure limits or guidelines	ppm

Notes:

* Can also analyze for in bulk, surface, and water samples.
CFU – colony forming units
m3 – cubic metres of air
mg – milligrams
ng – nanograms
ppm – parts per million
ml – millilitre
g – gram
**References used to generate Table 1 above are listed below[1-8]

References

1. Aerotech Laboratories Inc. IAQ sampling guide and chemical guide to IAQ. Phoenix, AZ: Aerotech Laboratories Inc; 2004.
2. EMLab P&K. IAQ pocket reference guide: mold, asbestos, bacteria, allergens, industrial hygiene, 6th ed. Marlton, NJ: EMLab P&K; 2011; Available from: https://www.emlab.com/s/services/pocketguide.html.
3. Goyer N, Lavoie J, Lazure L, Marchand G, Tessier V. Technical guide T-24. Bioaerosols in the workplace: evaluation, control and prevention guide. Montreal, QC: Institut de Recherche en Santé et en Sécurité due Travail du Québec (IRRST); 2001 Nov. Available from: http://www.irsst.qc.ca/en/-irsst-publication-bioaerosols-in-the-workplace-evaluation-control-and-prevention-guide-t-24.html.
4. Horner WE, Barnes C, Codina R, Levetin E. Guide for interpreting reports from inspections/investigations of indoor mold. J Allergy Clin Immunol. 2008 Mar;121(3):592-7.e7.
5. Kung'u J. Mold recognition, effective sampling strategies and results interpretation. Mississauga, ON: Mold and bacteria Consulting Laboratories Inc., and Wagner A. Golder Associates Limited; 2011. Available from: http://www.moldtraining.ca/courses/course/info.php?id=5.
6. Technical University of Nova Scotia. Microbial contamination in buildings. assessing, investigating and controlling. Course handout entitled: Chapter 3, investigating biocontamination problems, p. 53, Figure 17. Halifax, NS: Technical Univerity of Nova Scotia, Continuing Education Centre; 1995.
7. Yang CS. Assessment of fungal contamination in buildings Marlton, NJ: EMLab P&K; 2004. Available from: https://www.emlab.com/media/resources/Assessment-Fungal-Contamination-Buildings.pdf.
8. Yang CS, Heinsohn PA. Sampling and analysis of indoor microorganisms. New York, NY: Wiley; 2007. Available from: http://ca.wiley.com/WileyCDA/WileyTitle/productCd-0471730939.html.