



Aemtek Sampling Guides

Sampling for Airborne Fungal Spores to be analyzed by Quantitative Polymerase Chain Reaction (QPCR)

Scope

The scope of this procedure is for the identification and quantification of mold spores in air by QPCR. This method is a DNA-based technology and air samples are analyzed for a specific group of fungal species. QPCR analysis detects and quantifies viable and non-viable organisms.

Significance and Use

The use of QPCR is becoming more common as knowledge is acquired by IAQ professionals on the benefits of the technology. Testing by QPCR overcomes some of the problems encountered with traditional microbiological methods such as; extended incubation periods, required use of the correct media, overcrowding, and human error or misinterpretation.

This technology has a tremendous potential, particularly in highly specific niches such as: a). Post-remediation testing to assess remediation effectiveness, b). Species identification due to specific health effects and c). In cases when the investigation is targeted oriented to certain species.

Air sampling for QPCR is done using a 3-piece PCR cassette. This cassette is designed to capture large volumes of air, since overcrowding is rarely a problem for QPCR.

Some of the major advantages of this method are

- Quick turn around time (24 hrs or less)
- Highly sensitive
- Detect total bioaerosols, both viable and non viable
- Results are quantitative and qualitative to the species level

Some of the disadvantages include

- Limited to the availability of assays (species), may miss species that are not included in the test.
- Doesn't differentiate between viable and non-viable (pathogens need to be viable to cause disease).
- Cost.

Health and Safety Precautions

The use of protective equipment such as a mask, gloves, and protective clothing is recommended in highly contaminated environments or by sensitive individuals.

Apparatus and Supplies

Vacuum pump with flexible tubing.

Calibrated rotameter to calibrate the pump or, in sensitive cases, a primary pump calibration device such as a DryCal flowmeter (BIOS International Corp., Butler, NJ).

PCR cassette (3-piece, 37 mm cassette preloaded with a polycarbonate filter (pore size of 0.45 µm or 1.0 µm or equivalent).

Sampling Recommendation

- The number of samples per area will vary and depend on the specific case but enough samples should be collected to draw conclusions that are scientifically sound and at the same time economically feasible. In many cases where health issues are involved, larger numbers of samples or re-sampling may be required to obtain statistically sound results.
- Always include sampling of the outdoor environment as a source for comparison.
- It is recommended to include sampling of a non-problem area for comparison.
- Blank cassettes are recommended on a regular basis. These cassettes should be open in the testing area exactly in the same fashion as regular samples but no air is drawn through them.
- Make sure your rotameter or primary calibration device is working properly. Correct calibration of the pump is imperative to obtain good samples.
- Never use cassettes that appear opened or damaged.
- As any other sampling method for indoor environments, it is not recommended to utilize a single method to draw any conclusions in any indoor mold investigation. A combination of indoor sampling methods including direct microscopy, viable, surface samples etc. is recommended to obtain a better investigative profile.

Procedure

1. Calibrate pump. Air flow for PCR sampling is recommended to be between 3-10 L/minute.
2. Remove the red cap (outlet) from cassette and connect it pump.
3. Remove the blue cap (inlet) from cassette and direct it toward the sample site.
4. Take the air sample. Sampling time is almost theoretically unlimited, 500-3000 liters of air is the general recommendation (50 to 3000 minutes).
 - a. As an example at 5 L/minute, the sample should be taken for 100-600 minutes.
 - b. In some cases such as post-remediation testing, larger samples can be obtained to take advantage of the increased capacity offered by the method, increasing the validity of the data and making it more defensible
 - c. In dusty or other high particulate environments, smaller volumes are recommended to avoid clogging and possible interference from the dust or particulate.
5. At end of sampling time, replace both caps in cassette.
6. Label each cassette properly and record the amount of air collected in the Chain of Custody.
7. A blank cassette is recommended with every project as a control.
8. Pack and ship cassettes to laboratory to be analyzed, no ice is necessary. Packing material is recommended to avoid any damage during transportation.
9. Make sure to specify the fungus or group (package) of fungi to be tested for in the analysis.

Report

A report will be computer generated in a standardized report format. Quantification of fungi will be reported depending on fungal package tested. Quantification of fungal spores is reported as spores/m³ of sampled air.

Limit of Detection

Limit of detection has some variability depending on the species tested. As a general rule LOD will be between 10 and 100 spores per sample. Limit of detection may also be affected when PCR inhibitors are present in the sample however this normally is not a problem in air samples with exception of samples from very dusty environments.

Glossary

Limit of Detection (LOD): Smallest concentration of an analyte that can be detected by the measurement system

References

1. Introduction to food and airborne fungi. 2000. Edited by Samson *et. al.* Sixth Edition. 389 p.
2. Bioaerosols Assessment and Control. Edited by American Conference of Governmental Industrial Hygienists –ACGIH-. 1999
3. Haugland *et. al.* 2002 Journal of Microbiology Methods 50(2002 p. 319-323
4. Vesper S. J. *et. al.* 1999. Applied and Environmental Microbiology. July 1999. p. 3175-3181.

Sampling House Dust for Fungal Spores (direct microscopy), Viable (culturable) fungal species (culture method) or QPCR

Scope

The scope of this procedure is for the analysis of dust samples collected from suspected contaminated and dusty surfaces such as carpets, furniture, etc. Similar to bulk samples, this analysis can provide information on possible sources of microbial contamination, microbial toxins, allergens and irritants in the environment. Dust samples can be analyzed for quantification and identification of organisms by direct microscopy, culture, and/or QPCR.

Significance and Use

Dust samples are also considered bulk samples. Analyses of dust have been suggested to provide a good indication of cumulative exposures to mold since this matrix may serve as a reservoir of fungal contamination. Detection and quantification of fungal spores can be done by direct microscopy to obtain a general idea of the type and quantity of fungal organisms present in the sample regardless of viability. By the use of the culture method quantification and species identification of the viable (culturable) organisms present in the sample can be determined.

To collect dust samples, a 3-piece cassette or any other dust collector device such as a dust sock (attached to a conventional vacuum hose) may be used. A minimum of 0.1 g is necessary (a thimbleful is recommended).

Some of the benefits of this technique include:

- In the case of microscopic identification the fast turn around times (24 hrs). Ability to obtain a quick general idea of the type of microbial population present in sample.
- In the case of culture methods, species identification can be done in most or all the found organisms.

Some of the drawbacks of this technique are:

- In the case of microscopic identification, no differentiation is made of viable (culturable) versus non-viable (non-culturable) organisms. No species identification is possible.
- In the case of culture methods, long turn around times are necessary (7-10 days). Quantification may miss organisms that are non-viable (or non-culturable) which can still be a hazard as allergenic particles.

Health and Safety Precautions

The use of full protective equipment such as a mask, gloves, and protective clothing is recommended in highly contaminated environments or by sensitive individuals.

Apparatus and Supplies

Vacuum pump or any conventional household vacuum
Dust collector device (dust sock or equivalent)
Ziploc bags or equivalent

Sampling Recommendations

- The number of samples per area will vary and depend on the specific case but enough samples should be collected to draw conclusions that are scientifically sound and at the same time economically feasible. In many cases where health issues are involved, larger numbers of samples or re-sampling may be required to obtain statistically sound results.
- It is recommended to include sampling of a non-problem area for comparison.
- Blank cassettes/dust soaks are recommended on a regular basis. These cassettes should be open in the testing area exactly in the same fashion as regular samples but no sampling is drawn through them.
- Never use cassettes that appear opened or damaged.
- As any other sampling method for indoor environments, it is not recommended to utilize a single method to draw any conclusions in any indoor mold investigation. A combination of indoor sampling methods including direct microscopy, viable, surface samples etc. is recommended to obtain a better investigative profile.

Procedure

1. Mark a desired area to sample and vacuum in different directions. Make sure to have sufficient dust in each sample.
 - a. Make sure you have at least a thimbleful of dust. Samples with too many fibers or large materials should be avoided.
2. Label each sample and record the amount of sampled area in the Chain of Custody as well as the desired type of analysis to be performed.
3. Pack samples with wrapping material and ice (if culture method will be performed) and ship package *overnight* to Aemtek Inc.

Report

Results will be reported in a computer generated standardized report format. Names of fungi in report will be limited to the ones detected in sample with exception of *Aspergillus/Penicillium*, *Chaetomium*, *Cladosporium*, and *Stachybotrys* which will be mentioned in the report regardless of presence or absence in the sample. Quantification of fungal spores by microscopic analysis or QPCR is reported as number of spores per sample (spores/sample). For viable analysis results will be reported as Colony Forming Units CFU/sample.

Limit of Detection

The minimum limit of detection is one CFU per plate. LOD is determined taking into consideration the dilution and size of sample

Glossary

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References

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Sampling Surface Fungal Spores Using Swab and Bulk Sampling Methods And Analyzed by QPCR

Surface samples.

Scope

The scope of this procedure is for the analysis of surface samples by QPCR, which provides information as to if a suspect area is supporting microbial growth as a potential source of biological agents in the air. In addition, settled spores can also be determined on surfaces. Surface samples are analyzed by QPCR to quantify and identify to the species level. Identification is limited to certain fungal packages available.

Significance and Use

Surface sampling in IAQ investigation is usually done in combination with other methods such as air sampling. It is normally done when surface is a non porous (smooth) material and cutting it is undesirable or not an option (counter tops, poles, floors, etc). Surface sampling is most commonly done by wiping or swabbing a designated area. Bulk samples are also considered surface samples and are portions of materials (e.g. pieces of wallboard, duct lining, carpet, etc) that are tested to determine if they may contain, or be contaminated with, microbial agents. Bulk samples may look completely clean, show some discoloration, or clearly have microbial growth on it.

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This technology has a tremendous potential, particularly in highly specific niches such as: a). Post-remediation testing to monitor remediation effectiveness, b). Quick species identification when specific health effects are of concern, and c). In those cases when the investigation is targeted oriented to certain species.

Some of the major advantages of this method are

- Quick turn around time (24 hrs or less)
- Highly sensitive
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Some of the disadvantages include

- Limited to the availability of assays (species), may miss species that are not included in the test.
- Doesn't differentiate between viable and non-viable (pathogens need to be viable to cause disease).
- Cost.

Health and Safety Precautions

The use of protective equipment such as a mask, gloves, and protective clothing is recommended in highly contaminated environments or by sensitive individuals.

Apparatus and Supplies

Sterile swab (in sterile buffer) or sterile wipe
Ziploc plastic bag (or equivalent)
Knife or toll to be able to cut...

Sampling Recommendations

- If possible, it is recommended to use sterile gloves especially when highly contaminated surfaces are to be sampled.
- When using a swab/wipe make sure to use sufficient buffer to wet the swab/wipe to have a better recovery of spores present on the tested surface

Procedure

Swab/Wipe

1. Select the area to sample. It should be relatively non-porous material.
2. Swab the desired area thoroughly with a rolling motion with a moist sterile swab. Place swab in corresponding tube (zip lock bag are OK), and make sure it is completely sealed.
 - a. A sterile wipe can also be used to obtain the sample
 - b. Each sample must be in individual bags/tubes.
 - c. Some investigations also use what is called "tape lift". A piece of clear tape is imprinted on the desired surface and immediately placed either on a microscope slide or in a zip lock bag. This type of sample can only be analyzed for direct microscopy for mold identification and is not quantitative.
3. Label the sample and record the area sampled in the Chain of Custody. Make sure to specify the type of analysis needed.
4. Pack the sample with wrapping material and ice. Ship the sample *overnight* to Aemtek, Inc.

Bulk sampling

1. Obtain a piece (or pieces) of the desired material to be tested and place them *individually* in sealable plastic bags (zip lock bags work very well). The size of the sample will vary and some criteria should be taken into consideration.
 - a. Sample should be small so it can be easily packaged and transported to the laboratory.
 - b. If material shows different tones of discoloration or morphology, it is recommended to send either a large enough piece to represent the damage or take several samples to have a full representation of the possible different bacteria or fungi present in the damaged area.
2. Label each sample properly and fill up the Chain of Custody specifying the type of test required.
3. Pack sample(s) with wrapping material (use of ice is recommended but not critical) and send it *overnight* to Aemtek Inc

Report

A report will be computer generated in a standardized report format. Quantification of fungi will be reported depending on fungal package tested. Quantification of fungal spores is reported as #spores/sample or #spores/g.

Glossary

Limit of Detection (LOD): Smallest concentration of an analyte that can be detected by the measurement system

References

1. Introduction to food and airborne fungi. 2000. Edited by Samson *et. al.* Sixth Edition. 389 p.
2. Bioaerosols Assessment and Control. Edited by American Conference of Governmental Industrial Hygienists –ACGIH-. 1999
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