WHAT DO FUNGAL PCR RESULTS MEAN?

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Introduction

Sampling and testing for fungi is a common practice during an indoor environmental quality (IEQ) investigation, routine IEQ survey, or monitoring during mold remediation. The development and utilization of real-time polymerase chain reaction (RT-PCR) in detection and quantitation of fungi in the indoor environment has been made possible by a patented technology developed by the US Environmental Protection Agency (US-EPA). The technology offers several advantages over the traditional culturing and spore counting methods. The major advantages are: quick turnaround time, accurate fungal detection and identification, reproducible results, detection of fungi and fungal spores whether they are viable or not, quantitative and qualitative results, excellent quantitation sensitivity, and excellent detection sensitivity. Furthermore, the technology also allows sampling for a long duration (hours), minimizing the pitfalls of grab sampling (such as in culturable air samples and spore trap samples). On the other hand, consideration must be taken when performing PCR sampling and testing. There are currently no standards and guidelines regarding fungal PCR results of air samples. PCR is a fungal detection system, not an identification system. Fungal species must be expertly identified by a reputable mycologist. PCR analysis is specific to the types of fungi detected and can only determine the targeted species. There is no large database of fungi in the indoor air and indoor environments at this time.

Numeric standards and guidelines for fungi and airborne fungal spores are not likely to be available in the near future. Airborne fungi may change according to spatial and temporal variations. Fungi can multiply rapidly as long as nutrients and water are available. Without standards and guidelines, the current approach to the interpretation of PCR results of fungal samples relies on comparison of indoor vs. outdoor results and complaint vs. non-complaint area results.

This technical fact-sheet discusses how to interpret PCR results of fungal samples collected during an IEQ evaluation and investigation.

What are the advantages of using PCR analysis for fungi collected in the indoor environment?

There are several major advantages:

1. This is a patented technology developed by the US-EPA.
2. RT-PCR is based on the most advanced science on genetics.
3. It can provide quick turnaround time (a few hours to overnight).
4. It provides accurate fungal identification and detection.
5. It offers the advantages of both culturable (good fungal identification) and spore count (quick turnaround) methods.
6. It yields reproducible results.
7. It can detect fungal spores whether they are viable or not.
8. It yields quantitative and qualitative results.
9. It has excellent quantitation sensitivity.
10. It has excellent detection sensitivity.
11. It allows many hours of sampling, minimizing the pitfall of snapshot, grab sampling.

What are the disadvantages of using PCR analysis for fungi collected in the indoor environment?

The disadvantages of PCR analysis are:

1. It is relatively expensive. However, it is actually very cost effective when taking into consideration the advantage of sampling for many hours and a very large air volume.
2. This is not a test primarily designed for screening purposes. However, it can be used for screening important marker fungi of water damage. Specific species of fungi must be pre-determined prior to an analysis being performed. Culturable and spore counting methods are for screening purposes and produce results covering a broad spectrum of spores and fungi in the samples.
3. PCR cannot distinguish viable from non-viable spores/fungi.
4. There is currently no large database of indoor fungi available for PCR detection.

5. False positives and negatives may occasionally occur. Inhibitory contaminants in samples may cause no reaction and false negatives. Laboratory QA/QC as well as knowledgeable laboratory scientists can rule out the problems.
6. Not all fungal species can be detected by RT-PCR at this time. This will improve over time as we learn more about the fungal ecology indoors, and more primer/probe sets are developed.
7. Because of the extreme sensitivity, laboratory QA/QC becomes very important. A good laboratory must always have a QA/QC program ready.

However, the above disadvantages can be minimized or avoided if samples are analyzed by a reputable and credible laboratory staffed with knowledgeable mycologists.

When is PCR testing used in an IEQ assessment?
PCR testing can be used in all phases of IEQ assessment. Call and consult with our knowledgeable mycologists if you consider using PCR sampling and testing in your projects for the first time.

What is the sampling strategy for PCR testing?
The sampling strategy for PCR testing is generally no different from other fungal and mold sampling and testing. However, you can reduce your sample numbers (particularly air samples) because of the long sampling time and large air volume. In addition, PCR sampling and testing can replace both culturable and spore-trap sampling and testing. You may tailor your target fungal species to your project needs. For example, *Aspergillus fumigatus* and a few infectious aspergilli may be sampled for and determined by RT-PCR if the primary reason for sampling is for infection control in hospitals or health care facilities.

How do I use fungal PCR results?
For results of air samples, follow the steps below. However, you may skip steps that are not applicable.

1. Compare total concentrations from indoors, outdoors, complaint, and non-complaint areas. In general, indoor concentrations should be lower than those of outdoors. However, this may not always be consistent. Residential buildings, warehouses, schools and buildings with many entrances and openable windows, and buildings with HVAC systems with no filtration may have airborne fungal levels higher than or as high as those of outdoors. Results of non-complaint areas should consistently be lower than those of complaint areas.

2. Compare concentrations of each fungal species, indoors v. outdoors and complaint v. non-complaint areas. Concentrations of each fungal species from indoors and outdoors and complaint and non-complaint areas should be generally similar or lower in indoors and non-complaint area samples. For common outdoor fungi (such as *Cladosporium cladosporioides*), indoor levels of two times or higher than that of outdoors may be considered significant.

3. Determine the important fungi in your results. Most, if not all, fungi on our list are important indoor fungi. The following is important information on the fungi listed.

*Acremonium strictum:*This species has a world-wide distribution and is a common moisture-loving fungus. It can be found on wet, moist surfaces, or from wet walls (with acrylic paint), wallboard, wallpaper, or wood.

Alternaria alternata: This species is a common phylloplane and soil-borne fungus. It is in abundance in outdoor air during the crop harvesting season (fall). However, it is not uncommon to find this fungus growing on wet wallboard, wallpaper, wood, or textiles. If indoor concentrations of this fungus are at least twice those of outdoors, look into possible indoor source(s).

Aspergillus flavus/oryzae: Aspergillus flavus and *A. oryzae* are closely related and difficult to differentiate. However, *A. flavus* is a common contaminant of grains, nuts, and corn. It is not common on building materials but we have seen it on a few occasions. *Aspergillus flavus* may produce aflatoxins, which are carcinogens, causing liver cancer. In addition, it has been reported to cause aspergillosis, an infection of sinus cavities. *Aspergillus oryzae* is considered highly unusual in the US and from sources outside other than fermented foods, because it is found and primarily used in the fermented food industry. The current DNA sequence used cannot differentiate the two species at this time. The detection of *A. flavus/oryzae* by RT-PCR implies that it is more likely an *A. flavus* than an *A. oryzae* because of its ecology and geographic distribution.

*Aspergillus fumigatus:*This species is known for its growth at a wide temperature range and for causing most cases (80%) of aspergillosis. Because of its importance in causing aspergillosis in immune deficient people, it is included in most packages of PCR testing in our laboratory. Although it does not commonly grow in the indoor environment, we have found a few cases where the fungus actively grows indoors. It likes to grow in damp (very high humidity) and warm (37°C) conditions, or in places where wet organics are piled and stored. We have identified several environments where *A. fumigatus* is known to amplify. These include: basements with steam leaks, compost piles of mushroom farms, green yard-waste in recycling facilities, sawdust and wood chip piles in wood processing facilities and in paper mills.

Aspergillus niger: Spores of this species are relatively common in soil, plant litter, spices, and sun-dried plant products. It is found in floor, carpet, and mattress dust. Growth of this fungus on water-damaged paper products (such as newspaper, books, etc.) is not uncommon. It has been associated with aspergillosis and can cause infections in immune deficient people.

*Aspergillus ochraceus:*This is a xerophilic fungus and is occasionally found in indoor dusts. We have isolated this fungus from sweaty clothing.

Aspergillus sydowii: This fungus is a common aspergillus associated with water-damaged wallboard and wallpaper. It is more common in the south than *A. versicolor*. *Aspergillus versicolor* is much more common in the north. Both can be in a mixture from water-damaged materials. Although this fungus is not known to produce mycotoxins, cases of invasive aspergillosis caused by this fungus are known.

Aspergillus ustus: This fungus is an excellent indicator of water-damaged materials, particularly drywall and wallpaper. It is often found with *A. sydowii, A. versicolor*, and *Penicillium chrysogenum*.

Aspergillus versicolor: This fungus is a common aspergillus associated with water-damaged wallboard and wallpaper. It is known to produce mycotoxins, sterigmatocystin which is a precursor of aflatoxins. Both *A. sydowii* and *A. versicolor* are excellent indicators of water damage indoors. Although it has a minimum water activity (Aw) of 0.78 to 0.80, the optimal growth condition is 27°C and 0.98 Aw.

*Eurotium (Asp.) amstelodami:*This a strong xerophilic fungus. *Eurotium amstelodami* is a sexual state. The asexual state is called *Aspergillus vitis*. It is common in outdoor air. However, it grows indoors on materials stored in high relative humidity conditions.

Chaetomium globosum: This is an ascomycete (producing sexual spores in a sac-like structure called an ascus) possessing capability in degrading wood and paper products. This is one of the most common species in the genus *Chaetomium*. It is a moisture-loving fungus and an excellent indicator of water damage.

Cladosporium cladosporioides: This species is a common phylloplane and soil-borne fungus. It is in abundance in outdoor air during growing seasons. However, it does not tolerate high temperatures (>32°C) and its airborne concentrations may drop in extreme hot dry summer weather. It is occasionally found to grow on surfaces subjected to high relative humidity and with occasional condensation (such as fibrous glass insulation in the HVAC systems or the surface of oil-based or enamel paint). If indoor concentrations of this fungus are at least twice those of outdoors, look into possible indoor source(s).

Memnoniella echinata: This is another moisture-loving fungus. It is usually associated with water-damaged paper products, such as drywall paper and wallpaper. It is relatively common; however, the frequency is not as high as *Stachybotrys chartarum*. It is almost exclusively associated with *Stachybotrys chartarum* when it is isolated. These two fungi are closely related and both are known to produce mycotoxins.

Paecilomyces variotii: This fungus is often associated with composts and self-heating organics. Spores of this species are occasionally detected in air. It is commonly isolated from household dusts with known water damage. It has been known to grow on water-damaged wood (wood flooring to wood framing), damp sawdust, wood chips, etc.

Penicillium aurantiogriseum: This species of *Penicillium* is a good indicator of water-damaged drywall. It belongs to the *Penicillium aurantiogriseum* complex, including *P. polonicum, P. commune, P. viridicatum*, and *P. cyclopium*. It may also be mis-identified as another common *Penicillium* species, *P. chrysogenum*.

Penicillium brevicompactum: This *Penicillium* sp. is a xerophilic fungus common in carpet and house dusts. It has also been reported from damp walls and gypsum wallboard.

Penicillium chrysogenum: This species is extremely common in water damaged environments and, hence, in dust from water-damaged environments. It is frequently a dominant fungus in water-damaged gypsum wall board. It was previously known as *P. notatum*, the penicillin-producing fungus.

Penicillium glabrum: This is a common soil-borne penicilli of wide distribution. It is common in house dust, on damp walls, and from wood.

Penicillium variabile: This is another common soil-borne fungus and is found on damp building materials, including water-damaged drywall and paper products, and in house dust.

Scopulariopsis brevicaulis/fusca: Scopulariopsis brevicaulis is a common species found on damp walls, paper products (such as drywall paper and wallpaper), wood, and is common in house dust from damp buildings. *Scopulariopsis fusca* is a similar species. It is found in dust and has been isolated from straw and wood. The current DNA sequence used can not differentiate the two species.

Stachybotrys chartarum: This species is an excellent indicator of long-term water damage and is a moisture-loving fungus. It is a cellulolytic fungus and is considered a tertiary colonizer, particularly on paper products. Its primary substrates in a building are water-damaged drywall, wallpaper, ceiling tiles with cellulose components, paper backing of insulation, etc. It is believed that this species may include more than one taxon. The DNA sequence currently used in RT-PCR does not differentiate toxigenic from non-toxigenic strains.

Trichoderma viride/koningii: These two species are also excellent indicators of long-term water damage. They are cellulolytic species and are considered tertiary colonizers, particularly on paper and wood products. Their primary substrates in a building are water-damaged drywall, wallpaper, paper backing of insulation, and wood. The DNA sequence currently used in RT-PCR does not differentiate these two species. However, they can be easily distinguished under the microscope.

Ulocladium botrytis: This species is moisture-loving and an excellent indicator of water damage if detected in indoor samples. It can grow on water damaged wallboard, wallpaper, plaster wall, painted wall, wood, etc.

4. If *Aspergillus fumigatus, A. flavus,* and *A. niger* are detected in samples from hospitals and health care facilities, special attention is needed to locate the sources of these fungi. They can cause opportunistic infections in immune-deficient people.

5. Relate and correlate complaints, field observations, and laboratory results to determine if fungal contamination and growth occurs in the building or complaint area or not. Remember moisture and water are the critical factors in indoor fungal growth. If there is fungal growth, there must be a moisture or water problem nearby. Use the ecological information of each species to determine whether water damage is recent or long-term.

6. Keep in mind that RT-PCR only detects those fungi requested and is not a broad-spectrum screening test. There may be other significant fungi in water-damaged environments that are not detected.

7. For bulk, dust, wipe, or water samples, use the information in item 3 above. Also determine the dominant fungus (or fungi) in each sample. Dominance plus high concentrations can be used to determine whether a fungus or fungi are/were growing.

8. If results are used for quality assurance of a remediation project, determine what levels are acceptable. Focus on marker or indicator fungi, particularly those that have been identified growing in the project location.