PCR ANALYSIS FOR RAPID IDENTIFICATION AND QUANTITATION OF INDOOR FUNGI – THE FUTURE OF FUNGAL IDENTIFICATION

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Introduction

Recognition of fungi as the potential cause of health problems has raised great concerns about the health risks of those living and working in damp moldy homes and office buildings. Some are capable of causing allergic rhinitis (hay fever), hypersensitivity pneumonitis and allergic bronchopulmonary aspergillosis. Some fungi are capable of producing mcytoxins that pose a health concern to exposed individuals. Conventional fungal identification carried out by culture and microscopic analysis takes 7-14 days. A rapid and reliable detection and identification of the fungi is essential for facilitating better assessment of contamination and eventually the remediation process.

Researchers at the U.S. EPA have developed a new DNA-based realtime quantitative PCR technique. P&K has licensed this patented technology from U.S. EPA, and is currently offering this service for rapid identification and quantitation of indoor fungi.

What Is PCR Technology?

The explosion in molecular biology technology has initiated a new era in fungal identification. This cutting-edge technology for fungal identification is called polymerase chain reaction (PCR), a nucleic acid-based technology.
Every fungal species has its own-unique, characteristic genetic signature, which varies among species and can serve as a genetic fingerprint for their identification. The power of PCR is to amplify the selected sequence of genetic fingerprint to the level that it can be easily detected and quantitated.

At the forefront of PCR technology is called real time quantitative PCR which is currently used in our laboratory. In this system, a fluorogenic probe is added into the PCR amplification process. This probe will emit fluorescence, and the levels of accumulated fluorescence can be detected and quantitated in real time, during the amplification process. This fluorogenic probe is specially designed to bind only to the selected sequence of fingerprint, thus, providing additional target specificity in detection.

With the progression of cutting-edge PCR technology, we are now able to rapidly, quantitatively, and accurately detect contaminant fungal species in samples regardless of their culturability or viability.

Advantage of PCR Analysis

PCR technology not only overcomes the time-consuming process using conventional culture and microscopic analysis but also provides a sensitive, accurate and reliable analysis that you can count on. Furthermore, you can collect air samples in large air volume and for several hours. This will avoid the "snap shot" deficiency of most microbial air sampling of one to ten minutes. This also increases the quantitation sensitivity of the samples.

Following are the advantages of using PCR analysis.

- Quick turnaround time
- Accurate fungal identification
- Reproducible results
- Detection of fungal spores whether they are viable or not
- Quantitative and qualitative results
- Excellent quantitation sensitivity
- Excellent detection sensitivity

Convenient Packages for Analysis

To rapidly deliver meaningful results, we offer convenient packages that will aptly meet your needs at a reasonable cost. These packages are specially designed to look for marker or signature fungi. You will be impressed by our quality service and express delivery of reliable results.

PCR01
In this package, we provide a **broad coverage of 23 fungal species that may be found in a water-damaged environment**. This convenient package offers what you need most in conducting an assessment.

Acremonium strictum
Alternaria alternata
Aspergillus flavus/oryzae
Aspergillus fumigatus
Aspergillus niger
Aspergillus ochraceus
Aspergillus sydowii
Aspergillus ustus
Aspergillus versicolor
*Eurotium (Asp.) amstelodami**
Chaetomium globosum
Cladosporium cladosporioides
Memnoniella echinata
Paecilomyces variotii
Penicillium aurantiogriseum
Penicillium brevicompactum
Penicillium chrysogenum
Pencillium purpurogeum
Pencillium variabile
Scopulariopsis brevicaulis/fusca
Stachybotrys chartarum
Trichoderma viride/koningii
Ulocladium botrytis

PCR02
This package covers **15 fungal species found in water-damaged environments**.

Acremonium strictum
Aspergillus fumigatus
Aspergillus niger
Aspergillus sydowii
Aspergillus ustus
Aspergillus versicolor
Chaetomium globosum
Memnoniella echinata
Penicillium aurantiogriseum
Penicillium brevicompactum
Penicillium chrysogenum
Pencillium purpurogeum
Pencillium variabile
Stachybotrys chartarum
Ulocladium botrytis

PCR03
This package is designed for detecting the signature fungal species that are associated with **water damage**.

Acremonium strictum
Aspergillus versicolor
Chaetomium globosum
Memnoniella echinata
Penicillium aurantiogriseum
Penicillium chrysogenum
Stachybotrys chartarum
Ulocladium botrytis

PCR04
This package is specially designed for detecting **important indoor *Aspergillus* species**.

Aspergillus flavus/oryzae
Aspergillus fumigatus
Aspergillus niger
Aspergillus ochraceus
Aspergillus sydowii
Aspergillus ustus
Aspergillus versicolor
*Eurotium (Asp.) amstelodami**

PCR05
This package is specially designed for detecting important ***Aspergillus* species and *Penicillium* species** in the indoor environment.

Aspergillus ochraceus
Aspergillus sydowii
Aspergillus ustus
Aspergillus versicolor
Penicillium aurantiogriseum
Penicillium brevicompactum
Penicillium chrysogenum
Penicillium variabile

PCR06
We can also analyze a single specific fungus or a combination of your selection from the list below. The minimum numbers of samples for one species required is 8.
The following fungi are currently available for real time PCR analysis.

Acremonium strictum
Alternaria alternata
Aspergillus flavus/oryzae
Aspergillus fumigatus
Aspergillus niger
Aspergillus ochraceus
Aspergillus penicillioides
Aspergillus restrictus/caesillus/conicus
Aspergillus sydowii
Aspergillus ustus
Aspergillus versicolor
*Eurotium (Asp.) amstelodami**
Chaetomium globosum
Cladosporium cladosporioides
Memnoniella echinata
Paecilomyces variotii
Penicillium aurantiogriseum
Penicillium brevicompactum
Penicillium chrysogenum
Penicillium citrinum
Pencillium corylophilum
Penicillium expansum
Penicillium purpurogeum
Penicillium roquefortii
Penicillium variabile
Scopulariopsis brevicaulis/fusca
Stachybotrys chartarum
Trichoderma viride/koningii
Ulocladium botrytis
Wallemia sebi

PCR10
Wood decay dry rot detection and speciation: *Poria (Meruliporia) incrassata*, *Serpula lacrimans*, and *Serpula himantioides*

* *Eurotium* (*Aspergillus*) group includes *Eurotium amstelodami*, *E. herbariorum*, *E. chevalieri*, *E. rubrum*, and *E. repens*.

Sampling Protocol for QPCR Analysis

A. Liquid samples

a) Obtain sterile 15mL screw-cap tube for sampling. Obtain an extra tube as a field blank.
b) Keep the sampling tube closed until it is used. Fill up the tube with water sample.
c) Tightly cap the bottles. Make sure that water will not leak out during shipping and transporting.
d) Ship the samples on ice.

B. Bulk dust samples

a) Obtain 3-piece 37-mm cassettes, preloaded with 0.45 µm pore-size filters for sampling. Obtain an extra as a field blank.
b) A sufficient amount of dust is required for analysis, preferably 0.1 g or more.
c) Use clean masking tape to mark a surface area of your interest. A 12" x 12" area is suggested. However, you may increase the area or composite your samples from several 4" x 4" or 6" x 6" areas. Connect your filter cassette to the high volume pump with clean Tygon tubing of approximately 2-3 feet. Remove the top cover (open face) of the cassette and turn on the pump to sample by vacuuming. Sample within the marked area by vacuuming horizontally, vertically, and diagonally. When you finish sampling, turn the cassette up, cover it, and turn off the pump. Number your sample and record it on your chain of custody.
d) Other sampling devices, such as a vacuum cleaner interceptor bag, are also acceptable.

C. Air samples

a) Obtain 3-piece 37-mm cassettes, preloaded with 0.45 µm pore-size filters, for sampling. Obtain an extra as a field blank.
b) Sufficient air volume is required for analysis. A minimum of 600 L (based on 4-6 L/min for 120-180 min) or more (up to 1,800 L) is suggested. Close face sampling is recommended.

D. Wipe/Swab samples

a) Dip sterile cotton swab tip into sterile distilled water tube and moisten the cotton. Make sure the cotton is not too wet.
b) Use the wet cotton swab to wipe the suspected surface area and take sample. Label each sample clearly and record on COC.
c) Place the swab in a clean zip-lock bag or a sterile container.

E. Bulk Samples

a) Place the selected sample in a clean zip-lock bag or into a sterile container. Please provide special instructions if needed on your Chain of custody. Clearly label each bag.
b) You can also wipe the suspected area of bulk samples with the use of the sterile wet cotton swab as described in D wipe/swab samples. Record the area on the COC. Send them as wipe/swab samples as described above

F. Chain of Custody (COC)

a) Write the sample number on the container/cassettes and on COC sheet. Use a short distinctive number for each sample.
b) Complete all sample information on COC sheet, such as sampling date(s), air volume, time, location, your project or job number, purchase order number(s) for the job, your name, company name, phone and fax number. Keep your own record and send a copy with samples to the laboratory.

G. Shipping samples

a) For liquid samples, place containers in a clean plastic bag then put into an insulated box with blue ice or reusable ice packs to maintain the temperature between 2 to 8 °C. **Do not use ice cubes or dry ice.** Stuff the box with foam chips to provide sufficient cushion and seal the box securely for shipping.
b) Place air and dust samples in plastic bags and then in a cardboard box. Securely seal and tape the bag for shipping.
c) Send samples to the laboratory by overnight express carrier. Call and inform the laboratory. Take holidays into consideration.

References:

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