Sampling Guidelines for QPCR
Microbial Investigations

Introduction

The Quantitative Real-Time Polymerase Chain Reaction assay (QPCR) utilizes unique genetic sequences that correspond to a particular mold species. Extracting the DNA and probing for these unique sequences allow us to confirm the presence of a particular mold species. Utilizing the Sequence Detection System 7000 by ABI, the rate of recognition and amplification of these genetic sequences is reported and viewed in real-time, which results in quick, accurate identification and quantitation of mold present in a given sample.

The use of QPCR in the investigation of microbial contamination associated with water intrusion will dramatically change how investigations are planned and performed. Some basic shortcomings of traditional sampling are overcome with the use of QPCR. The advantages of PCR include:

- Speciation and quantification of mold within a day
- Instrumental Identification and quantitation, eliminating analyst subjective bias.
- Use of a single sampling train for performing air sampling.
- Adaptability to the collection of personnel samples.
- Smoothing of temporal variability through the use of long-term sampling.
- No refrigeration or incubation of media required.
- Differentiation and species identification of *Penicillium* and *Aspergillus* in a timely and cost-effective manner.

Since QPCR provides species-specific results by instrumental methods, obstacles traditionally associated with microscopic identification are removed. Traditional non-culturable methods cannot identify mold to species and cannot differentiate *Penicillium* from *Aspergillus*. Species-specific data can be obtained quickly using QPCR. Currently many investigators utilize culturable techniques for the sole purpose of discerning the presence of *Aspergillus* and *Penicillium* species and for comparisons of environments at the species level. Given the widespread presence of *Aspergillus* and *Penicillium* in both problem and non-problem buildings, as well as during pre- or post-remediation activities, identifying and quantifying the relevant species in a more cost effective, timely fashion is one of the most significant uses of QPCR.

Sampling Protocols

I. Bulk Sampling

Bulk sampling may be classified as either:

A. Three dimensional substrates
B. Surface dust samples

All sampling, handling, and shipping of three dimensional substrates and surface samples can be done without the need for refrigeration.

A. Three dimensional samples:

A typical representative sample of the material may be submitted in a new, clean double zip-lock bag. The material will be appropriately sub-sampled in the laboratory. The results will be reported as spore-equivalents detected in the sub-sample; the number of spore-equivalents reported will be semi-quantitative only.

B. Surface Samples:

Forensic Analytical has validated QPCR analysis of micro-vacuum dust samples. The analysis of swabs and wipes has not yet been validated; currently, we are not recommending swab or wipe sampling for QPCR analysis.

Dust (Micro-vacuum) Samples:

Dust samples may be collected using a sampling train similar to that used for air sampling. A high-volume air sampler set at a flow rate between 5 to 15 lpm is typically employed. A 0.45-0.8 micron polycarbonate, PVC or MCE filter cassette attached by standard Tygon tubing to the pump completes the sampling train. Samples may be collected either open or closed faced (a short piece of Tygon tubing with a 45 degree cut edge is attached to as a “vacuum hose” to the nipple of the cap of the cassette) depending on the investigation and surface.

There is no maximum loading requirement, but we recommend sampling 6 square feet of carpet and a larger area of uncarpeted surfaces. For carpet, sample a total of 6 square feet, preferably six sections of 1 square foot each. Vacuum each square foot for 1 minute at 15 lpm or 3 minutes at 5 lpm. Templates of one square foot are commercially available. For uncarpeted surfaces, collect as much surface dust as feasible. For clean surfaces, this may entail vacuuming a significant fraction of the surface. The results will be reported as spore-equivalents per gram of dust.

II. Air Sampling

QPCR analysis can be performed on air samples collected on pre-loaded filter cassettes. All sampling handling and shipping of cassettes can be done without the need for refrigeration.

Commercially available cassettes containing 0.45 micron polycarbonate filters should be used for analysis by QPCR. Flow rates and sample durations may vary, but Forensic Analytical has validated the method indoors and outdoors at 15 lpm. For best quantitation, Forensic recommends 8-hour @ 15 lpm samples indoors and at least 4-hour @ 15 lpm samples outdoors. The results will be reported as spore-equivalents per liter of air.

For additional information and updates on panel development, or to request a specific test regimen, please visit our Real-time PCR website information page at www.forensica.com/docs/mic/PCR.html, or contact us at (800) 827-3274