

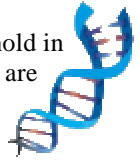


## Species Identification and Quantification of Molds Using Real-Time PCR\*

\* Based on methods developed and licensed by the United States Environmental Protection Agency

### What is Real-Time PCR?

- A **FAST, ACCURATE, and SENSITIVE** DNA-based analytical method for identifying and quantifying molds to the species level.
- Looks for the presence of DNA sequences that are unique to a particular mold species.
- Utilizes a DNA sequence detection system to monitor the presence and concentration of a specific mold in “REAL TIME”. As a mold-unique sequence is detected and amplified, fluorescent signal molecules are simultaneously released and measured. No fluorescence = no target mold.



### Real-Time PCR is a DNA-based analytical method. What is DNA?

- DNA is a nucleic acid that carries the genetic information that is unique to every organism. DNA sequences determine individual hereditary characteristics
- DNA can be found in every cell in every living (or previously living) organism. For example, humans have DNA in their skin cells and blood cells and molds have DNA in their conidial cells (spores) and mycelial cells (hyphae).

### Can you please compare PCR analysis to the cheaper Spore Trap analysis (e.g. Air-O-Cell, LARO-100, Cyclex, Cyclex-D, Burkhard) for mold identification?

- Air-O-Cell and PCR are both **FAST** tests that provide you with information regarding quantity (# of spores) and identity of molds present in your samples. They can both be completed in a day.
- Air-O-Cell analysis gives you **genus** names (ex. *Stachybotrys*) of approximately 20 to 30 different molds and tells you if you have any *Aspergillus/ Penicillium*-like spores (Asp/Pen spores are very difficult to distinguish in spore traps). PCR analysis gives you **genus** and **species** names (ex. *Stachybotrys chartarum*) of all the molds you choose to look for.
- Air-O-Cell analysis is **subjective**, depending on the expertise of the analyst. PCR analysis is **not subjective** since it is a DNA-based test.
- Neither Air-O-Cell nor PCR analysis can tell you if the spores in your sample are viable or not (alive or dead).
- Air-O-Cell analysis can be impossible to do if the sample is overloaded due to extended sampling time. PCR analysis can be done with as few as a single spore or as many as a billion spores.

### Can you please compare PCR analysis to culture analysis for identification of molds to the species level?

- Both PCR and culture analysis can provide you with **species** names of molds in your sample.
- Culture analysis requires us to grow the molds for 7-14 days in order to isolate and adequately see the presence of distinguishing physical characteristics on molds. PCR analysis does not require us to grow the molds because it only analyzes DNA. Since DNA is present at any stage, analysis can be performed immediately.
- Traditional techniques to identify cultured molds to the species level are subjective and rely heavily on the expertise of the analyst, while PCR technology is based on conserved DNA sequences and are not biased.
- Culture analysis can miss slow growing molds, non-culturable molds, or molds that are present in very low quantities because faster growing molds can out-compete them for nutrients and space on culture media. PCR analysis can detect molds that are present in very low concentrations as well as extremely high concentrations.
- Culture analysis can reveal which molds are viable. PCR cannot distinguish between viable and non-viable molds.

## Comparison of PCR analysis to spore trap and culture analysis

	Spore Traps	Cultures	PCR
<b>Speed</b>	1 day	7-14 days	1 day
<b>Identification</b>	Genus level of all identifiable mold spores (no ID of hyphae)	Genus and/or species level of viable spore-producing molds (no ID of hyphae)	Genus and species level of client selected molds
<b>Quantification</b>	Spores	Colony Forming Units	Spores*
<b>Accuracy</b>	GOOD	BETTER	BEST
<b>Viability</b>	Cannot be determined	Can be determined	Cannot be determined
<b>Sampling Time</b>	Limited due to possibility of overloading sample	Limited due to possibility of overloading sample	No restrictions

\*In PCR, a hyphal fragment with 1 nucleus is reported as 1 spore equivalent.

### Why is species identification of molds important?

- Not all species within a single genus of fungi are pathogenic or have the ability to produce mycotoxins.

### Why is the identification of non-viable molds as well as viable molds important?

- Non-viable (i.e. dead) molds, like viable molds, can still be allergenic as well as toxic.

### What are the best reasons for choosing Real-Time PCR?

- It is very **fast**. You do not need to wait for the mold to grow and produce distinctive fungal structures. Analysis can be performed immediately on any type of sample – air, swab, bulk, or water.
- It is extremely **accurate**. It looks for unique species-specific DNA sequences.
- It is very **sensitive**, with almost no minimum or maximum detection limit.

***REAL-TIME PCR***  
***FAST · ACCURATE · SENSITIVE***



## REAL-TIME PCR SAMPLING GUIDE

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**All PCR sampling supplies are provided by EMSL free of charge with PCR analysis.**

Real-Time PCR is an excellent complement to your current sampling strategies. If you currently use spore traps to reveal the genus names of molds in indoor environments, you can also submit real-time PCR samples to quickly determine the species names of any significant molds that were found on the spore traps. If you use culture techniques to determine the identity of viable organisms, you can use real-time PCR to determine the species names of those organisms sooner. Whatever method you prefer, you can be assured that your results will be accurate and reliable.

### **Air Samples**

1. Obtain a 3-piece PCR air/dust-sampling cassette from EMSL.
2. Remove the upper (blue) and lower (red) plugs of the cassette.
3. Attach a high volume pump to the cassette through the lower opening.
4. Sample as much air as desired through the upper opening. There is no upper limit to sampling time.
5. Record the VOLUME of air sampled and ship cassette to EMSL. No refrigeration is needed.

### **Dust Samples**

1. Obtain a 3-piece PCR air/dust-sampling cassette from EMSL.
2. Remove the upper (blue) and lower (red) plugs of the cassette.
3. Attach a small piece of tubing to the upper opening. Cut a 45 degree angle at the end of the tubing.
4. Attach a high volume pump to the cassette through the lower opening.
5. Begin collecting dust through the upper tubing. There is no upper or lower limit to sampling.
6. Ship the cassette to EMSL. No refrigeration is needed.

### **Swabs**

1. Obtain a sterile swab from EMSL to collect specimen.
2. Wearing gloves, remove swab from packaging material.
3. Swab the desired area thoroughly, rolling the swab lightly back and forth over sampling area. Be sure to record the AREA sampled.
4. Insert the swab into the tube and firmly close cap. Ship the swab to EMSL. No refrigeration is needed.

### **Bulk samples**

1. Obtain a sterile swab and follow directions as described above for swabs.
2. Alternatively, you may submit a piece of the bulk sample for analysis.

### **Water/liquid Samples**

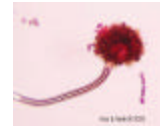
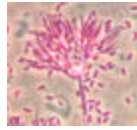
1. All water samples should be taken in sterile sampling containers, which our laboratory can provide upon request.
2. Fill container with 100 ml of water, or as much water as possible and seal securely to prevent leakage during transport.
3. Transport sample using ICE PACKS to EMSL.

### **Culture Plates**

If you are interested in fast speciation of your culturable (viable) molds in your samples, you may combine PCR with culture analysis. Simply ask the laboratory to hold your cultures for follow-up analysis and request the desired PCR organism package.



## REAL-TIME PCR ORGANISM PACKAGES



\*\* You may choose more than one package for each sample submitted for PCR analysis.

### PCR - Water Damage Packages

**Water damage 20.** The following 20 organisms are commonly associated with water damaged environments.

**24 hours - \$420**

**48 hours - \$280**

**Add \$25 for dust samples**

*Stachybotrys chartarum*

*Ulocladium botrytis*

*Aspergillus nidulans*

*Aspergillus versicolor*

*Penicillium expansum*

*Alternaria alternata*

*Penicillium aurantiogriseum*

*Aspergillus flavus*

*Cladosporium cladosporioides*

*Penicillium chrysogenum*

*Aspergillus niger*

*Cladosporium sphaerospermum*

*Aspergillus fumigatus*

*Aspergillus ochraceus*

*Trichoderma viride*

*Chaetomium globosum*

*Penicillium brevicompactum*

*Paecilomyces variotii*

*Acremonium strictum*

*Aspergillus ustus*

**Water damage 15.** The following subset of 15 organisms is commonly associated with water damaged environments.

**24 hours - \$360**

**48 hours - \$240**

**Add \$25 for dust samples**

*Stachybotrys chartarum*

*Ulocladium botrytis*

*Aspergillus versicolor*

*Penicillium expansum*

*Penicillium aurantiogriseum*

*Aspergillus flavus*

*Penicillium chrysogenum*

*Aspergillus niger*

*Aspergillus fumigatus*

*Aspergillus ochraceus*

*Chaetomium globosum*

*Penicillium brevicompactum*

*Acremonium strictum*

*Aspergillus ustus*

*Aspergillus nidulans*

**Water damage 10.** The following subset of 10 organisms is commonly associated with water damaged environments.

**24 hours - \$270**

**48 hours - \$180**

**Add \$25 for dust samples**

*Stachybotrys chartarum*

*Chaetomium globosum*

*Aspergillus versicolor*

*Acremonium strictum*

*Penicillium aurantiogriseum*

*Ulocladium botrytis*

*Penicillium chrysogenum*

*Penicillium expansum*

*Aspergillus fumigatus*

*Aspergillus flavus*

**Water damage 5.** The following subset of 5 organisms is commonly associated with water damaged environments.

**24 hours - \$150**

**48 hours - \$100**

**Add \$25 for dust samples**

*Stachybotrys chartarum*

*Aspergillus versicolor*

*Penicillium aurantiogriseum*

*Penicillium chrysogenum*

*Aspergillus fumigatus*

### PCR - *Stachybotrys* package

**Stachybotrys 2.** The following two *Stachybotrys* species are associated with water damaged environments. Both species are known mycotoxin producers.

**24 hours - \$110**

**48 hours - \$75**

**Add \$25 for dust samples**

*Stachybotrys chartarum*

*Stachybotrys (Memnoniella) echinata*



## PCR - *Aspergillus* packages

**PCR – *Aspergillus* 10.** The following 10 *Aspergillus* species are commonly associated with contaminated indoor environments.

**24 hours - \$270**

**48 hours - \$180**

**Add \$25 for dust samples**

*Aspergillus fumigatus*

*Aspergillus niger*

*Aspergillus versicolor*

*Aspergillus ustus*

*Aspergillus flavus*

*Aspergillus parasiticus*

*Aspergillus ochraceus*

*Aspergillus sydowii*

*Emericella (A.) nidulans*

*Eurotium (A.) amstelodami*

**PCR - *Aspergillus* 5.** These 5 *Aspergillus* species are associated with mycotoxin production and/or aspergillosis, a fungal infection of lungs.

**24 hours - \$150**

**48 hours - \$100**

**Add \$25 for dust samples**

*Aspergillus fumigatus*

*Aspergillus versicolor*

*Aspergillus flavus*

*Aspergillus ochraceus*

*Aspergillus parasiticus*



## PCR - *Penicillium* packages

**PCR - *Penicillium* 10.** The following 10 *Penicillium* species are commonly found in contaminated indoor environments.

**24 hours - \$270**

**48 hours - \$180**

**Add \$25 for dust samples**

*Penicillium chrysogenum*

*Penicillium brevicompactum*

*Penicillium expansum*

*Penicillium corylophilum*

*Penicillium roquefortii*

*Penicillium simplicissimum*

*Penicillium aurantiogriseum*

*Penicillium crustosum*

*Penicillium citrinum*

*Penicillium fellutanum*

**Penicillium 5.** These 5 *Penicillium* species are important because they are known mycotoxin producers.

**24 hours - \$150**

**48 hours - \$100**

**Add \$25 for dust samples**

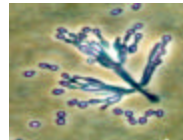
*Penicillium chrysogenum*

*Penicillium expansum*

*Penicillium roquefortii*

*Penicillium aurantiogriseum*

*Penicillium citrinum*



## Create your own package

Choose from the following 30 organisms to create your own package of species. More species will continue to be added over time and with client interest.

**24 hours - \$110 per species**

**48 hours - \$75 per species**

**Add \$25 for dust samples**

*Acremonium strictum*

*Alternaria alternata*

*Aspergillus amstelodami*

*Aspergillus flavus*

*Aspergillus fumigatus*

*Aspergillus niger*

*Aspergillus nidulans*

*Aspergillus ochraceus*

*Aspergillus parasiticus*

*Aspergillus sydowii*

*Aspergillus ustus*

*Aspergillus versicolor*

*Chaetomium globosum*

*Cladosporium cladosporioides*

*Cladosporium sphaerospermum*

*Paecilomyces varioti*

*Penicillium aurantiogriseum*

*Penicillium brevicompactum*

*Penicillium chrysogenum*

*Penicillium citrinum*

*Penicillium corylophilum*

*Penicillium crustosum*

*Penicillium expansum*

*Penicillium fellutanum*

*Penicillium roquefortii*

*Penicillium simplicissimum*

*Stachybotrys chartarum*

*Stachybotrys echinata*

*Ulocladium botrytis*

*Trichoderma viride /konigii*

