

REAL-TIME

PCR

IDENTIFICATION OF MOLD AND BACTERIA



*Fast,
Accurate...
Species Level
ID and Quantification*



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REAL-TIME PCR SAMPLING GUIDE

ALL PCR SAMPLING SUPPLIES CAN BE OBTAINED DIRECTLY FROM EMSL.

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 vacuum 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 vacuum 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.

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).

COMPARISON OF PCR ANALYSIS TO SPORE TRAP AND CULTURE ANALYSIS

	Spore Traps	Cultures	PCR
Speed	1 day	7-14 days	1 day
Identification	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
Quantification	Spores	Colony Forming Units	Cells*
Accuracy	GOOD	BETTER	BEST
Viability	Cannot be determined	Can be determined	Cannot be determined
Sampling Time	Limited due to possibility of overloading sample	Limited due to possibility of overloading sample	No restrictions

*Cells include spores and hyphae.

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.

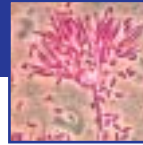
WHAT ARE THE BEST REASONS FOR CHOOSING REAL-TIME PCR?

- 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 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

Stachybotrys chartarum
Aspergillus versicolor
Penicillium aurantiogriseum
Penicillium chrysogenum
Aspergillus fumigatus
Chaetomium globosum
Acremonium strictum

48 hours - \$280

Ulocladium botrytis
Penicillium expansum
Aspergillus flavus
Aspergillus niger
Aspergillus ochraceus
Penicillium brevicompactum
Aspergillus ustus

Add \$25 for dust samples

Aspergillus nidulans
Alternaria alternata
Cladosporium cladosporioides
Cladosporium sphaerospermum
Trichoderma viride
Paecilomyces variotii

WATER DAMAGE 15. The following subset of 15 organisms is commonly associated with water damaged environments.

24 hours - \$360

Stachybotrys chartarum
Aspergillus versicolor
Penicillium aurantiogriseum
Penicillium chrysogenum
Aspergillus fumigatus

48 hours - \$240

Chaetomium globosum
Acremonium strictum
Ulocladium botrytis
Penicillium expansum
Aspergillus flavus

Add \$25 for dust samples

Aspergillus niger
Aspergillus ochraceus
Penicillium brevicompactum
Aspergillus ustus
Aspergillus nidulans

WATER DAMAGE 10. The following subset of 10 organisms is commonly associated with water damaged environments.

24 hours - \$270

Stachybotrys chartarum
Aspergillus versicolor
Penicillium aurantiogriseum
Penicillium chrysogenum
Aspergillus fumigatus

48 hours - \$180

Chaetomium globosum
Acremonium strictum
Ulocladium botrytis
Penicillium expansum
Aspergillus flavus

Add \$25 for dust samples

WATER DAMAGE 5. The following subset of 5 organisms is commonly associated with water damaged environments.

24 hours - \$150

Stachybotrys chartarum
Aspergillus versicolor
Penicillium aurantiogriseum
Penicillium chrysogenum
Aspergillus fumigatus

48 hours - \$100

Add \$25 for dust samples

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

Stachybotrys chartarum

48 hours - \$75

Stachybotrys (Memnoniella) echinata

Add \$25 for dust samples



PCR - ASPERGILLUS PACKAGES

ASPERGILLUS 10. The following 10 Aspergillus species are commonly associated with contaminated indoor environments.

24 hours - \$270

Aspergillus fumigatus
Aspergillus versicolor
Aspergillus flavus
Aspergillus ochraceus

48 hours - \$180

Emericella (A.) nidulans
Aspergillus niger
Aspergillus ustus

Add \$25 for dust samples

Eurotium (A.) amstelodami
Aspergillus sydowii
Aspergillus parasiticus

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

24 hours - \$150

Aspergillus fumigatus
Aspergillus versicolor
Aspergillus flavus
Aspergillus ochraceus
Aspergillus parasiticus

48 hours - \$100

Add \$25 for dust samples



PCR - PENICILLIUM PACKAGES

PENICILLIUM 10. The following 10 Penicillium species are commonly found in contaminated indoor environments.

24 hours - \$270

Penicillium chrysogenum
Penicillium expansum
Penicillium roquefortii
Penicillium aurantiogriseum
Penicillium citrinum

48 hours - \$180

Penicillium brevicompactum
Penicillium corylophilum
Penicillium simplicissimum
Penicillium crustosum
Penicillium fellutanum

Add \$25 for dust samples

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

24 hours - \$150

Penicillium chrysogenum
Penicillium expansum
Penicillium roquefortii
Penicillium aurantiogriseum
Penicillium citrinum

48 hours - \$100

Add \$25 for dust samples



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

Acremonium strictum
Aspergillus fumigatus
Aspergillus parasiticus
Chaetomium globosum
Penicillium aurantiogriseum
Penicillium corylophilum
Penicillium roquefortii
Ulocladium botrytis

48 hours - \$75 per species

Alternaria alternata
Aspergillus niger
Aspergillus sydowii
Cladosporium cladosporioides
Penicillium brevicompactum
Penicillium crustosum
Penicillium simplicissimum
Trichoderma viride

Add \$25 for dust samples

Aspergillus amstelodami
Aspergillus nidulans
Aspergillus ustus
Cladosporium sphaerospermum
Penicillium chrysogenum
Penicillium expansum
Stachybotrys chartarum
Aspergillus flavus
Aspergillus ochraceus
Aspergillus versicolor
Paecilomyces variotii
Penicillium citrinum
Penicillium fellutanum
Stachybotrys echinata



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