Microbial QPCR Guide:
Segment - Fungi

Assured Bio Labs, LLC | (865) 813-1700 | info@assuredbio.com

TABLE OF CONTENTS

What is DNA Mold Testing?

DNA Analysis

DNA Testing allows for accurate and rapid detection of a wide variety of organisms. Assured Bio Labs is at the forefront of DNA testing being one of the first labs in the United States to be accredited for molecular detection of molds and bacteria by AIHA.

What Makes DNA Testing Better

• DNA testing is fast: most analyses can be run with same day turn around for additional cost.
• DNA testing is flexible; we can sample from just about every sample matrix and can run many analyses from a single sample and are also able to design custom testing for clients per their request.
• DNA testing is accurate; some tests are accurate down to one cell per sample type. It also eliminates human error due to no actual human interaction in the analysis. This makes it a great choice for issues that may end up in court.

MSQPCR

Mold Specific Quantitative Polymerase Chain Reaction (MSQPCR) is the umbrella assay that describes 36 mold DNA targets developed and patented by the U.S.E.P.A. National Exposure Research Laboratory program. DNA targets have the ability to probe a mixed sample of extracted DNA and bind only to a single mold species' DNA. Once bound, the target emits fluorescent light that is detected by specialized laboratory instruments to provide species ID and the spore or spore equivalent concentration in a sample. Examples of analyses that incorporate MSQPCR targets include the ERMI, SIM, and SIAM. The ERMI was the first comprehensive DNA analysis developed by the EPA for residential use. The sample matrix is dust. The dust must be collected from carpet using a single dust collector sample cassette from the master bedroom and common living area in the home. Dust provides insight into the mold history of a home. When following the EPA's ERMI protocol for sample collection an ERMI score is provided. The ERMI score reports the mold burden of a home as compared to an index developed by the EPA from a segment of homes across the country.

The SIM incorporates the same 36 species targets as the ERMI, and is used for other sample types that do not conform to the ERMI protocol. For example, the ERMI specifies dust must be collected from carpet in a home. The SIM has no dust restrictions, and may be collected from any surface in any occupied space (home, school, manufacturing etc.). Moreover, swabs may be substituted for dust cassettes. Note: tape lifts are incompatible with any DNA test. The sticky residue prevents proper

DNA extraction in the laboratory. While the ERMI and SIM provide insight into the historical mold burden of the built-environment, the SIAM detects and quantifies airborne mold species that are present in the here and now, and is crucial for mold exposure assessment.

The SIAM uses Assured Bio Labs patented M-TRAP® cassette. The M-TRAP® functions like a spore trap, but with superior 3-D capture efficiency (95-98% better than spore traps). Moreover, the M-TRAP® cassette is made for rapid species ID and quantification with DNA. It may be used with any standard IAQ sampling pump and 1/4" tubing.

Other Testing

Assured Bio offers a wide range of bacterial testing. Bed bugs are a nuisance and difficult to remove once established. Assured Bio's DNA bed bug test prevents infestations with early detection before colonies establish. DNA detection is also available for Histoplasma/Cryptococcus (avian pathogens) and the infectious amoeba Acanthamoeba). Assured Bio provides DNA testing for many organisms. Check out the comparison list below. Moreover, if there is an organism not on the list, but required for a project, please contact us. Our scientists routinely develop custom DNA assays for clients and industry.

Turnaround Time

Standard turnaround time for DNA analyses is 2 to 3 business days from sample receipt. Same day turnaround may be available at additional cost of $100 per sample and $125 for weekend processing depending on your specific needs. Inquire for details.

Custom Sample Processing and Reporting

In some cases, Assured Bio can accommodate custom sample processing and reporting requests. Atypical sample types and custom report requests may incur an additional processing fee. Inquire for details. Assured Bio provides the opportunity for clients to create custom panels to suit their needs. Please call the laboratory with all inquiries for more information or to have a custom panel created with special pricing.

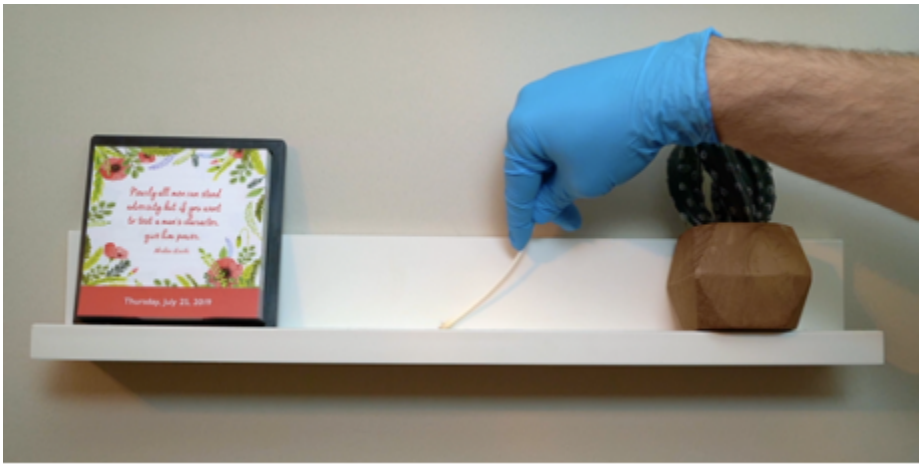
Collecting MSQPCR Samples

Collecting dust samples

Collecting dust samples can get you a look at the mold history in the home. If you follow the EPA's ERMI protocol, you will receive an ERMI score which is useful to compare one home against the mold burden in the average home. A SIM (Survey of Indoor Molds), which uses all the same technology as the ERMI, can be used for other sample types that do not conform to the ERMI protocol. This analysis can be used with either a dust collector or swab.

(See the next page to view sampling options)

Sterile Swab



Collecting samples using a sterile swab is one of the most common ways to collect DNA mold samples. This method involves collecting from four dust reservoirs per room. Examples analyses are: **SIM** (Survey of Indoor Molds) or **The Big 2**.

How to use: Utilizing a sterile swab, the inspector is able to collect up to 2 rooms. Collecting samples from two rooms then makes the analysis a screen of both rooms. Both rooms will be sampled with the same swab. Once the inspector has decided which room/rooms they wish to collect their sample from, they will then label them on the Chain of Custody form. Common rooms to collect from are the master bedroom and the living room. Using the cotton end of the swab, the inspector will **collect four dust reservoirs per room (the same swab will be used for all dust reservoirs)**. DO NOT SAMPLE VISIBLE MOLD GROWTH (Wiping the swab on visible growth will result in high mold counts that will not provide an accurate representation of the room's indoor air quality). Gently wipe the cotton end on each dust reservoir until dust material is visible on the swab head. It is important to rotate the cotton end over the dust reservoir to maximize the amount of dust collected.
Call the lab for more detailed instructions.

Dust Cassette

Samples collected with the dust cassette may be deemed most useful when the area of collection is from a carpet. Example analyses are: **ERMI** (Environmental Relative Moldiness Index) or **ARMI** (American Relative Moldiness Index).

How to use: In order to collect samples using a dust cassette, ensure that you have masking tape, a vacuum with a hose attachment and carpet. Using masking tape, start out by taping a 6 by 3 foot rectangle on your carpet. You will then remove the bottom white cap and begin vacuuming the entirety of the rectangle for five minutes. Avoid making contact with the inside of the rectangle. If contact must be made, we recommend the inspector to wear gloves and/or protective coveralls to prevent contamination of the sample.
Call the lab for more detailed instructions.

Collecting air samples

In order to conduct air samples using DNA technology, the inspector will need to collect the sample with the M-TRAP® Air Sampling Cassette and WhisperCare® Continuous Air Monitoring System.

(See the next page to view sampling options)

The M-TRAP® Air Sampling Cassette

The M-TRAP® is a unique air sampling cassette developed specifically for the rapid capture and analysis of airborne fungi, bacteria, and viruses using state-of-the-art DNA & RNA analysis. The M-TRAP® embodies the next generation of capture technology for residential and occupational mold exposure assessment. It excels where spore traps and agar impactions fall short: spore capture efficiency and turnaround time. Example analyses are: **SIAM** (Survey of Indoor Airborne Molds) or **Total Fungi**.

How to use: In order to collect fungal samples using the M-TRAP, inspectors will collect samples using the blue label cassettes. M-TRAP® may be connected via ½" tubing commonly used with air sampling pumps that are currently on the market. The pump's flow rate should be set at 15 liters per minute prior to use and the sample should be collected for 10 minutes yielding a sample volume equivalent to 150 liters of air.
Call the lab for more detailed instructions.

Source: Molecular Entrapment White Paper

The WhisperCare® Continuous Air Monitoring System

The WhisperCare® is a continuous air monitoring system designed to test facilities for mold, bacteria and viruses using DNA & RNA analysis. With its low decibel output, your facility can continuously collect air samples without disturbing occupants from their day-to-day routine. Using a longer capturing rate increases the accuracy of your data when tracking down the source of contamination. The WhisperCare® can be used across the commercial, industrial, manufacturing, healthcare and residential sectors. Example analyses are: **SIAM** (Survey of Indoor Airborne Molds) or **Total Fungi**.

How to use: To collect samples using the WhisperCare, place each WhisperCare® in the desired sampling locations and apply your gloves. Without touching the white filter membrane (cotton-end), remove your M-TRAP® cassettes from the sterile housing (close and keep the sterile housing cases for when sampling is completed). Place the M-TRAP® cassettes onto the fittings of each WhisperCare® securely. Plug in your WhisperCare® to begin sampling. Document the WhisperCare® locations, the serial numbers of the M-TRAP® cassettes attached to it and sign the date and time each sample collection started on your Chain of Custody form. Once sampling is complete, apply your gloves. Carefully remove your M-TRAP® cassettes from each WhisperCare®, document the time they were removed on the Chain of Custody, and place them back into their sterile housing (plastic case). You will then open up a new pair of M-TRAP® cassettes and begin the process over again.
Call the lab for more detailed instructions.

DNA Testing Panel Comparisons

DNA Panel Comparisons

Each MSQPCR testing panel contains a variety of different fungal testing options. Users are also able to test for individual fungi species of fungi or add an individual species of fungi to an already existing testing panel. Inspectors or other health and safety professionals are eligible for price breaks. Contact our lab for pricing information.

Disclaimer: Each fungal species listed with an “x” are testable molds for that specific panel and will provide quantitative results. Any fungal species listed with “P/A” in the listed column are testable molds for that specific panel and will provide a presence/absence result (positive or negative).

(See the next page to view comparisons)

Organism	ERMI	SIAM	ARMI	SIM	Hertsmi-2	BIG 2	BIG 2 +Cheatomium	CAP 2	CAP 4	CAP 8	CAP 14	CAP 15	Pathogenic Fungus Panel	Aspergillosis Assesment	Penicillium Assessment	Avian Pathogen Panel	Pathogenic Candida Panel	Total Fungi
Aspergillus fumigatus	x	x		x						x	x	x	x	x				
Aspergillus ochraceus	x	x	x	x								x		x				
Aspergillus restrictus	x	x	x	x										x				
Aspergillus sclerotiorum	x	x		x										x				
Aspergillus unguis	x	x		x										x				
Aspergillus versicolor	x	x		x	x						x	x	x	x				
Aspergillus penicillioides	x	x	x	x	x						x	x		x				
Cladosporium sphaerospermum	x	x		x														
Eurotium (Aspergillus) amstelodami	x	x	x	x						x	x	x						
Penicillium purpurogenum	x	x		x										x				
Stachybotrys chartarum	x	x		x	x	x	x			x	x	x						
Aspergillus flavus	x	x		x							x	x	x	x				
Aspergillus niger	x	x	x	x						x	x	x	x	x				
Aspergillus sydowii	x	x	x	x										x				
Aureobasidium pullulans	x	x		x														
Chaetomium globosum	x	x	x	x	x		x				x	x	x					
Paecilomyces variotii	x	x	x	x									x					
Penicillium brevicompactum	x	x		x					x	x	x	x			x			
Penicillium corylophilum	x	x		x											x			
Penicillium crustosum	x	x		x											x			
Penicillium glabrum	x	x		x											x			
Penicillium variabile	x	x		x											x			
Scopulariopsis brevicaulis	x	x		x									x					
Scopulariopsis chartarum	x	x		x														
Trichoderma viride	x	x		x							x	x	x					
Wallemia sebi	x	x	x	x	x						x	x						
Acremonium strictum	x	x		x									x					
Alternaria alternata	x	x	x	x									x					
Clasdosporium cladosporioides svar. 1	x	x	x	x				x	x	x	x	x						
Clasdosporium cladosporioides svar. 2	x	x		x														
Cladosporium herbarum	x	x	x	x														
Aspergillus ustus	x	x		x									x	x				
Epicoccum nigrum	x	x		x														
Mucor amphibiorum	x	x		x									x					
Penicillium chrysogenum	x	x	x	x					x	x	x	x			x			
Rhizopus stolonifer	x	x		x														
Geo	x	x		x														
Penicllium/Aspergillus Group						x	x											
Aureobasidium pullulans								x	x	x	x	x						
Histoplasma capsulatum																(P/A)		
Cryptococcus neoformans																x		
Candida auris																	x	
Candida albicans																	x	
Total Fungi																		x

Example Reports

Example Report: SIAM

Sampling Method: M-TRAP
Number of Samples: 2

Species Identification of Airborne Molds (SIAM)

Using Mold Specific Quantitative Polymerase Chain Reaction (qPCR)

228 Midway Lane, Suite B
Oak Ridge, Tennessee 37830
Toll Free: (866) 547-1727
Local: (865) 813-1700
Fax: (865) 813-1705
Email: info@assuredbio.com
www.assuredbio.com

Inspector:	Bruce Wayne	**Date Collected:**	9/13/2020
Project Name:	Wayne Manor	**Date Received:**	9/14/2020
Project Number:	98765	**Date Reported:**	9/16/2020
Assured Bio Identifier:	BW091520-99	**Analyst(s):**	C. Kent

Selected References

Meklin, T. M., R. A. Haugland, T. Reponen, M. Varma, Z. Lummus, D. Bernstein, L. J. Wymer and S. J. Vesper. 2004. Quantitative PCR analysis of house dust can reveal abnormal mold conditions. Journal of Environmental Monitoring 6:615-620.

Vesper, S. J., C. McKinstry, C. Yang, R. A. Haugland, C. M. Kercsmar, I. Yike, M. D. Schluchter, H. L. Kirchner, J. Sobolewski, T. M. Alltan and D. G. Dearborn. 2006. Specific molds associated with asthma in water-damaged homes. Journal of Occupational and Environmental Medicine 48:852-858.

Meklin T et al. 2007. Comparison of mold concentrations quantified by MSQPCR in indoor and outdoor air sampled simultaneously. Science of the Total Environment 382 (1):130-134.

Accreditation

Assured Bio Labs, LLC is accredited by the American Industrial Hygiene Association Laboratory Accreditation Programs, LLC (AIHA-LAP, LLC; Lab ID # 183867) in the Environmental Microbiology accreditation program for "qPCR - Mold Specific qPCR" Fields of Testing as documented by the Scope of Accreditation Certificate and associated Scope. AIHA-LAP, LLC accreditation complies with the ISO/IEC Standard 17025:2005 requirements, but this does not imply ISO certification or registration."

Limitations

ASSURED BIO LABS, LLC MAKES NO WARRANTIES AND EXPRESSLY DISCLAIMS THE IMPLIED WARRANTIES OF MERCHANTABILITY AND FITNESS FOR PURPOSE. INSPECTOR ACKNOWLEDGES THAT ASSURED BIO LABS, LLC HAS NOT INSPECTED THE SUBJECT PROPERTY AND THAT THE INSPECTOR IS SOLELY RESPONSIBLE FOR CHOOSING THE LOCATION OF SAMPLE COLLECTION. ASSURED BIO LABS, LLC SHALL NOT BE LIABLE TO INSPECTOR FOR ANY INCIDENTAL, CONSEQUENTIAL, SPECIAL OR PUNITIVE DAMAGES OF ANY KIND OR NATURE, INCLUDING, WITHOUT LIMITATION, ANY DAMAGES TO PROPERTY OR PERSONAL INJURY WHETHER SUCH LIABILITY IS ASSERTED ON THE BASIS OF CONTRACT, TORT, OR OTHERWISE, EVEN IF ASSURED BIO LABS, LLC HAS BEEN WARNED OF THE POSSIBILITY OF SUCH LOSS OR DAMAGE. UNDER NO CIRCUMSTANCES SHALL ASSURED BIO LABS, LLC BE LIABLE FOR DAMAGES UNDER OR ARISING OUT OF THIS REPORT IN AN AMOUNT EXCEEDING THE AMOUNT PAID BY THE INSPECTOR TO ASSURED BIO LABS, LLC FOR THIS ANALYSIS AND REPORT. THIS REPORT IS FOR THE SOLE USE OF THE INSPECTOR AND CREATES NO THIRD PARTY BENEFICIARIES OR RIGHTS HEREUNDER.

Abbreviations

ND – None Detected

Methods of Analysis

Assured Bio Labs uses the following methods for the MSQPCR analysis: CD 23: Data Reporting for MSQPCR Testing, CD 143: Preparation, Processing, and Analysis of MSQPCR Samples, CD 225: Bead Based DNA Extraction

Reporting Limits

Method Detection Limit (MDL): The American Industrial Hygiene Association defines this term in AIHA-LAP, LLC Policy Document – Module 9 as "The minimum concentration of an analyte that, in a given matrix and with a specific method, has a 99 percent probability of being identified, qualitatively or quantitatively measured, and reported to be greater than zero."

Reporting Limit (RL): The American Industrial Hygiene Association defines this term in AIHA-LAP, LLC Policy Document – Module 9 as "The lowest concentration of analyte in a sample that can be reported with a defined, reproducible level of certainty."

Values less than one will be rounded up to one per reported unit.

Method Detection Limits (in Spores)

Astrc – 1.346, Aaltr – 42.41, Anigr – 0.3142, Aflav – 30.23, Afumi – 0.6582, Aochr1 – 851.5, Apeni2 – 0.3027, Arest – 4.372, Asclr – 0.1648, Asydo3 – 29.95, Aungu – 0.4572, Austs2 – 0.09001, Avers2-2 – 38.18, Apull – 0.0938, Cglob – 0.7785, Cclad1 – 0.0403, Cclad2 – 1.049, Cherb – 0.0233, Cspha – 0.0328, Eamst – 0.0897, Enigr – 0.0051, Muc1 – 0.02438, Pvari2 – 0.09652, PenGrp2 – 5.199, Pbrev – 7.549, Pchry – 4.897, Pcory – 1.662, Ppurp – 0.5208, Pvarb2 – 7.758, Pspin2 – 13.19, Rstol – 0.6516, SCbrv – 0.02846, SCchr – 0.6106, Stac – 0.1616, Tviri – 15.21, Wsebi – 7-111

Reporting Limit Calculations

Unless otherwise stated in comments, the following equations are used to calculate the reporting limit per sample: MTrap RL – MDL × (1000/L sampled)

Summary of Species Identification of Airborne Molds

The Species Identification of Airborne Molds (SIAM) is a collection of assays employing Mold Specific Quantitative Polymerase Chain Reaction (MSQPCR) technology. This technology was developed by the United States Environmental Protection Agency and is based upon more than a decade of research and development for indoor air fungi. MSQPCR, itself, is simply a method by which fungal DNA is copied. The action of copying the DNA makes it possible for a fluorescent probe specific to each species to be detected. As more DNA is copied, more fluorescent light is produced. Perhaps most importantly, the entire process can be completed rapidly. The simplicity of this system translates into an analysis that is both robust and reliable. No other method currently used in indoor air quality can compare to the speed and reproducibility of MSQPCR.

This report is designed by Assured Bio Labs, LLC to appeal to industrial hygienists and other highly trained and experienced individuals. As opposed to other panels offered for MSQPCR analysis, this panel carries with it no score that indicates relative moldiness. Instead, the SIAM panel emphasizes individual species quantifications and offers a granular assessment of the numbers of each fungal species or group of species detected. These raw numbers are emphasized over a score because they will be used by the hygienist in forming recommendations and strategies for remediation.

To aid the hygienist receiving this report, a list of species descriptions has been compiled and is provided with each report. Each description includes a brief statement relative to the natural and indoor ecology of the species, and observations of toxicity and/or pathogenicity are included when this information is known. Several commonalities can be noted for these species. Most, if not all, species of fungi occurring indoors are soilborne. Almost all of the species in this panel have worldwide distributions, rendering them useful indicators of indoor conditions in any location. Additionally, mycoses (fungal infections) have been documented for most of the species. Many mycoses occur in individuals with weakened or suppressed immune systems, and the presence of most species does not ensure infections to occur in occupants of homes. However, infections can and do occur in healthy people.

Species Descriptions

***Acremonium strictum* (Astrc)**
This species is a common inhabitant of soils worldwide and can be isolated from plant surfaces, fuel and fuel filters. Important in an indoor context, *A. strictum* is found widely in the atmosphere and is common observed on food and moist indoor surfaces (e.g. humidifiers). It is possible that moldy homes can show greater numbers of this species in winter months. *Acremonioum strictum* has caused infections in chemotherapy and transplant patients. Infections of blood, cerebrospinal fluid, eye, pulmonary, peritoneal, toenail and fingernail have been reported for this species, although they appear to be relatively rare. However, reports of infections by species of the genus *Acremonium* appear to be on the rise.

***Alternaria alternata* (Aaltr)**
This fungus can be found throughout the world on and in plants, soils, textiles and foods. *Alternaria alternata* is among the most commonly observed molds in indoor environments, and its spores are released diurnally. It is found frequently in moist/humid areas such as watertanks and humidifiers. However, this species is also quite common in dry areas including dust from floors and mattresses. This species produces several allergens and mycotoxins (tenuazonic acid and altertoxins). Members of the genus *Alternaria* are known to cause asthma, sinusitis and infections of the eyes, ears and skin.

***Aspergillus flavus/oryzae* (Aflav)**
Isolates of *Aspergillus flavus* and *A. oryzae* are morphologically indistinguishable. *Aspergillus flavus* can be found virtually anywhere on Earth and has been isolated from dry areas in Chile, antarctic lakes, humidifiers, plants, insects, animals, leather, feathers, cotton fabrics, paintings, etc. Concentrations of *A. flavus* in American soils are more dense in the southern U.S. *Aspergillus flavus* can produce aflatoxins under some circumstances. Aflatoxin B_1 is the most potent carcinogen (cancer-causing agent) known, and lethal doses of this compound are known to be extremely low in mice. In humans, *A. flavus* can cause pulmonary aspergillosis and weakened patients can suffer from cutaneous, nasal and cerebral infections.

***Aspergillus fumigatus, Neosartorya fischeri* (Afumi)**
Neosartorya fischeri is a heat-tolerant fungus that is common in soil and fruits and occasionally causes human infections. *Aspergillus fumigatus* is also heat-tolerant and found worldwide. *Aspergillus fumigatus* it is particularly dense in agricultural soils but is commonly isolated from house dust, garbage, compost, potted plants, humidifiers and HVAC systems, as well. More importantly, *A. fumigatus* is isolated commonly from human patients. In healthy humans, *A. fumigatus* is not a pathogen. However, this species can cause severe infections in humans with suppressed immune systems (e.g. those with pre-existing illnesses or taking immunosuppressants). In such individuals, spores that are inhaled are not attacked efficiently by the host's immune system, and the spores could germinate and begin to invade host tissues.

***Aspergillus niger/awamori/foetidus/phoenicis* (Anigr)**
Species detected by this assay are morphologically similar and difficult to distinguish without molecular techniques, such as PCR. *Aspergillus awamori* is widespread in soils and on plants, and it has been used extensively for industrial applications and for food preparation. Some isolates of *A. awamori* have been found to produce the mycotoxin known as ochratoxin A, and it is possible that this fungus can cause subcutaneous infections. *Aspergillus foetidus* and *A. phoenicis* are soil fungi that are likely involved in natural decomposition. *Aspergillus niger* is a fungus that can be found in house dust, mattress dust. *Aspergillus niger* can also contaminate foods such as spices and onions. Importantly, *A. niger* is allergenic and can cause inner/outer ear infections and sinus infections.

***Aspergillus ochraceus/ostianus* (Aochr1)**
These species of *Aspergillus* can be found indoors and on foodstuffs (e.g. coffee and paprika). Both species produce ochratoxin A, but *A. ostianus* can also produce aflatoxin.

***Aspergillus penicillioides* (Apeni2)**
This fungal species is common in very dry conditions and can be isolated from dried fruits, spices, archives, furniture, carpets, house dust and clothing. It is also associated with dust mites and is known to be allergenic.

***Aspergillus restrictus/caesillus/conicus* (Arest)**
Aspergillus restrictus is a fungus that is more likely to be isolated in cool and dry climates, which could explain its frequent occurrence in house dust. All three of these species are considered medically important, although infections are not widely documented.

***Aspergillus sclerotiorum* (Asclr)**
This species is found in tropical and subtropical soils across the world. *Aspergillus sclerotiorum* can produce ochratoxins and is known to cause infections of the ear, toenails and fingernails.

Species Descriptions (Continued)

***Aspergillus sydowii* (Asydo3)**
This species is found in soils worldwide and has been isolated from plants, seeds, foods, leather, textiles and uranium mines. *Aspergillus sydowii* can produce mycotoxins know as sydowic acids and can cause fingernail and toenail infections and invasive aspergillosis.

***Aspergillus unguis* (Aungu)**
Very little is known about this fungal species. However, it has been found to cause fingernail and toenail infections.

***Aspergillus ustus* (Austs2)**
It is likely that *A. ustus* is one of the most widely spread species of *Aspergillus*. It has been isolated from diverse soils from around the world, salt marshes, estuaries, foods, bat caves and uranium mines. Sporulation of *A. ustus* is stimulated by light. This species produces several mycotoxins and has been responsible for endocarditis and infections of the lungs and skin. It is possible that infection by *A. ustus* is nosocomial, but diagnoses of this mycosis are rare.

***Aspergillus versicolor* (Avers2-2)**
As are most aspergilli, *A. versicolor* is extremely widespread in nature. However, this species tends to occupy the coldest regions of *Aspergillus* distributions, as well as deserts, peat bogs, estuarine sediments, compost, linoleum, chipboard, paintings, cheeses, spices, stored grains, house dust, mattress dust and rotting military equipment in the tropics. This species is extremely xerophilic and common in indoor environments, where its growth can cause moldy odors. *Aspergillus versicolor* is known to produce a carcinogenic compound known as sterigmatocystin. *Aspergillus versicolor* is allergenic, and mycoses of this species include osteomyelitis and infections of the auditory canal, fingernails and toenails.

***Aureobasidium pullulans* (Apull)**
This fungal species is ubiquitous. Isolations are most common from plant leaves but have been successful from such diverse environments as humidifiers, house dust, mattress dust, forest soils, sand dunes, peat bogs, estuarine sediments, marine sediments and seawater. In British homes, airborne spores of this species increase sharply in winter months. Interestingly, *A. pullulans* does not appear to require high levels of nutrients commonly needed by other environmental microbes. This species is also extremely sensitive to heat, and can be found in high-humidity areas (e.g. window frames and bathrooms). This fungus is implicated rarely in human infections of the eyes and skin, and *A. pullulans* infections can be found in blood.

***Chaetomium globosum* (Cglob)**
This fungus is isolated commonly from soil, decaying plants, seeds, food, estuarine environments and marine sediments. It has particular notoriety as a soft rot fungus and can be found on decaying wood, explaining its occurrence in indoor environments following water damage. In fact, *C. globosum* can be found growing on wallpaper in homes with extensive water damage. Sporulation of this fungus tends to occur more readily under dark conditions, and the spores produced are very resistant to desiccation. While not particularly allergenic itself, its presence appears to enhance the allergic response of individuals to other allergens (e.g. pollen). This species has caused invasive lung infections, subcutaneous infections and fingernail and toenail infections. The genus *Chaetomium* appears to be emerging as important fungal pathogens.

***Cladosporium cladosporioides* svar. 1 and svar. 2 (Cclad1 & Cclad2)**
These two organisms are not currently recognized as individual species, and they cannot be differentiated using standard microscopic techniques. DNA sequencing projects seeking to devise rapid identification methods for fungal pathogens detected distinct DNA sequences in this species, and each is now recognized as a "sequevar." In essence, they cannot be identified correctly without the use of DNA-based technology, such as this quantitative PCR technique. Both sequevars represent the most common saprobe in the environment. This species generates many more spores under moist conditions than in dry conditions. *Cladosporium cladosporioides* is distributed worldwide in soils, air, house dust, mattress dust, on dairy products, textiles, food, plants, many aquatic environments, wood pulp and feathers. This species is allergenic and can form fungal balls in lungs, skin infections, keratitis, sinusitis, and infections of spinal fluid, fingernails and toenails.

***Cladosporium herbarum* (Cherb)**
This is a very common fungus in nature, and it can be isolated from dead/dying plants, soil, food, wheat, textiles, floor dust, mattress dust, seawater, uranium mines and paint. In fact, it is possible that *C. herbarum* is the most common *Cladosporium* in air samples and appears to be more prevalent in summer months in British homes. However, this species was found to cause food spoilage at refrigeration temperatures. *Cladosporium herbarum* was found to be strongly allergenic and produces an endotoxin that has similar health effects to that produced by *Stachybotrys chartarum*.

Species Descriptions (Continued)

***Cladosporium sphaerospermum* (Cspha)**
As with most species of *Cladosporium*, *C. sphaerospermum* is common worldwide. This species can be isolated from plants, soil, food, paint, textiles, insulation, floor dust, mattress dust, humidifiers and from humans and other animals. Spores of this species are difficult to distinguish from those of *C. cladosporioides* microscopically, but DNA analyses easily distinguish them. *Cladosporium sphaerospermum* is one of the most commonly isolated indoor air fungi. This species is allergenic and has caused documented bronchial lesions and subcutaneous skin infections.

***Epicoccum nigrum* (Enigr)**
One of the most commonly isolated indoor fungi, *E. nigrum* is also widely distributed in nature. It can be found growing in and on soils, sand, dead/decaying plant tissue, saline environments, textiles and moldy paper. At this time, *E. nigrum* is not known as a pathogen, but this species can cause skin allergies.

***Eurotium* (*Aspergillus*) *amstelodami/chevalieri/herbariorum/rubrum/repens* (Eamst)**
This assay identifies a group of closely related *Eurotium* species. Most molds isolated from indoor environments are asexual species, however some also reproduce sexually. To discern these two modes or reproductive states, mycologists have devised a unique terminology. The term "anamorph" describes those molds that reproduce asexually; whereas, the term "teleomorph" describes molds that reproduce sexually. Anamorphic or asexual molds do not need a partner to reproduce, they produce their spores similar to budding yeast cells and do so on a grand scale; millions if not billions of spores are produced in a short period of time (24-48 hrs). Anamorphic reproduction is an evolutionary strategy that fires the conflict between humans and molds in homes and buildings; just add water to building materials, and mold will seem to appear out of nowhere and rapidly colonize the damp substrates. Teleomorphic molds, however, must find and fuse with a compatible partner or strain in order to produce spores sexually. Hence, teleomorphic molds are rare relative to anamorphic molds because the paring of compatible strains in the environment is governed by the laws of probability, and the probability of two microscopic strains meeting at any given location is remote. However, some teleomorphic species tend to commonly occur indoors.

The most common teleomorphic genus is *Eurotium*. *Eurotium* species are perhaps the most abundant sexually reproducing molds found indoors. The key to *Eurotium's* success lies in genetics, for *Eurotium's* asexual counterpart is *Aspergillus*. *Aspergillus* species produce enormous flushes of spores. Hence, *Aspergillus* spores are extremely common, especially in a water compromised building. The relative abundance of *Aspergillus* spores dramatically increases the probability that two compatible aspergilli strains will meet and fuse to form a teleomorphic *Eurotium* species. Thus, *Eurotium* has become an important mold genus, one that should not be ignored during indoor air quality assessments. This genus is xerophilic and has the ability to germinate and colonize substrates having minimal water activity. *Eurotium* is also a common food spoilage organism.

Eurotium has been implicated in several health maladies. Anamorphic forms of *Eurotium* produce various mycotoxins. Farmer's lung disease (FLD) is caused mainly by repeated exposure to moldy hay colonized by *Eurotium* species. *Eurotium* may be a respiratory allergic in susceptible individuals and can cause adverse health effects in children who attend school in buildings damaged by moisture.

***Mucor amphibiorum/circinelloides/hiemalis/indicus/mucedo/racemosus/ramosissimus* and *Rhizopus azygosporus/homothalicus/microsporus/oligosporus/oryzae* (Muc1)**
The species of mold represented in this assay are all members of a broad class of fungi known as Zygomycetes. Zygomycetes are primitive but fast growing fungi. They are widely distributed in terrestrial environments, where they break down plant debris in soil. However, many species are common environmental contaminants that can cause food spoilage, and a few are pathogens of plants, insects and humans. By definition, all pathogenic zygomycotic species will grow at 37 °C, with the possible exception of the *M. circinelloides*.

The common genera that infect humans include *Rhizopus*, followed by *Mucor*, *Rhizomucor*, *Absidia*, *Cunninghamella* and *Syncephalastrum*. Underlying diseases in humans include cancer and leukemia, antibiotic or prednisone use, diabetes, deferoxamine and desferrioxamine therapy, transplantation, burn wounds and the associated forms of immunosuppressive therapies. The most common clinical form of zygomycosis is rhinocerebral disease followed by pulmonary, cutaneous/subcutaneous, gastrointestinal and disseminated disease. *Mucor amphibiorum* has not been reported in human infections. *Mucor circinelloides* has been reported as a rare cause of cutaneous infections in humans. *Mucor hiemalis* has been reported from a few cases of human cutaneous infection. *Mucor indicus* (synonym: *M. rouxii*) has been reported from human gastric and pulmonary infections, a case of necrotizing fasciitis and reports of hepatic infection in a bone marrow transplant recipient who had ingested contaminated medicine. *Mucor racemosus* has been infrequently reported as a causative agent of animal and human zygomycosis. *Rhizopus microsporus* accounts for 10-15% of reported human cases and has been implicated in cellulitis, cutaneous infection, zygomycosis, and gastrointestinal infections. However, rhinocerebral forms of *R. microsporus* are rare. *Rhizopus oryzae* (synonym: *R. arrhizus*) is the most common causative agent of zygomycosis, accounting for 60% of the reported culture positive cases and nearly 90 percent of the rhinocerebral form of infection.

Species Descriptions (Continued)

***Paecilomyces variotii* (Pvari2)**
This fungus is known to be heat resistant and can, therefore, be found most commonly in warm and arid environments. It is also very common in air, animal feed, seawater, wood pulp in paper mills, creosote-treated wood, walls, wallpaper, house dust, compost, leather, optical lenses, synthetic rubber, photographic paper, moldy cigars, ink, optical lenses, PVC and kerosene. *Paecilomyces variotii* has been known as a pathogen in birds and mammals but also appears to be an important human pathogen and infects the heart, lungs, bones, spleen and soft tissue.

***Penicillium brevicompactum/stoloniferum* (Pbrev)**
Penicillium stoloniferum is a relatively rarely occurring fungus found in soils and foods. *Penicillium stoloniferum* commonly attacks poinsettias in Switzerland greenhouses but is not currently recognized as a health threat. *Penicillium brevicompactum* is a common species worldwide and indoors, occurring in fruit juices, fresh herbs, wall paper, wood, paint, potted plants (particularly strong association), soils, floor dust, mattress dust, caves, freshwater and uranium mines. *Penicillium brevicompactum* can be xerophilic but sensitive to high-salt conditions. This species also inhibits the growth of several species of soil bacteria, possibly through production of its several mycotoxins (e.g. ochratoxin). *Penicillium brevicompactum* can be strongly allergenic, but it has not been implicated widely in human disease. However, *P. brevicompactum* has been isolated from a dog with fungal pneumonia and a deep organ infection in a human.

***Penicillium chrysogenum* (Pchry)**
This species is found worldwide but has earned most notoriety from its production of penicillin. In addition to soil distributions, it can be isolated from foods, plants, floor dust, mattress dust, wood, wall paper, paint, gypsum (as in wall board) artwork and occasionally optical lenses. It is considered a good indicator of water intrusion. Although this species is highly allergenic and can produce mycotoxins, *P. chrysogenum* is not considered a common health risk. Nonetheless, infections of the ears, eyes, heart tissue, skin and cerebrospinal fluid have been documented.

***Penicillium corylophilum* (Pcory)**
This species is widely distributed, but it is found more frequently in warm climates. Isolations have been successful from soil, textiles and various foods. This species is thought to be relatively xerophilic and is likely more common in low-humidity conditions, probably explaining their isolation from wood and paint. At this time, *P. corylophilum* does not appear to be a human pathogen.

***Penicillium crustosum/camemberti/commune/echinulatum/solitum* (PenGrp2)**
Penicillium crustosum is a common food contaminant, particularly common in seeds, nuts and apples. *Penicillium crustosum* produces potent neurotoxins (penitrems and roquefortine) that can cause muscular tremors in individuals eating contaminated foods. *Penicillium camemberti* is a mold commonly found in cheeses (camembert cheese) and occasionally meats, where it can produce low levels of the mycotoxin cyclopiazonic acid. *P. commune* is commonly found indoors and on cheeses and meats. *Penicillium commune* has been documented in pulmonary infections in dogs and can produce cyclopiazonic acid and possibly nephrotoxins. *Penicillium echinulatum* is found most frequently on foods containing oils (e.g. margarine and cheese) but is also found indoors. *P. echinulatum* is capable of producing tremorgenic mycotoxins (territrems). *Penicillium solitum* is commonly isolated from foods such as hard cheeses and some meats. *Penicillium solitum* can produce mycotoxins (viridicatins) on such foods but does not appear to cause diseases in humans.

***Penicillium glabrum/lividum/purpurescens/spinulosum/thomii* (Pspin2)**
Penicillium glabrum is a commonly occurring indoor fungus, but it can also be found contaminating foods (particularly fruit and fruit products) and growing in compost and aggressively on computer diskettes in high humidity. *Penicillium glabrum* also grows well on the corks of wine bottles and elicits allergic responses in individuals that work with wine corks. *Penicillium lividum* is a relatively rare and non-pathogenic species of *Penicillium* and occurs mostly in northern latitudes. *Penicillium purpurescens* is a common inhabitant of soils and indoor environments (particularly greenhouses). *Penicillium purpurescens* does not appear to be an overt pathogen, but it can be found in feed potentially toxic to poultry. *Penicillium spinulosum* is distributed worldwide and is usually found associated with forest soils, flour-based foods and fruit products. *Penicillium spinulosum* can grow on wet plasterboard, and such growth can yields mycotoxin production, the health effects of which are under debate. *Penicillium thomii* is widely distributed in soils of temperate environments. *Penicillium thomii* does not appear to be pathogenic, given current data.

***Penicillium purpurogenum* (Ppurp)**
This is another example of a *Penicillium* with a worldwide distribution in soils. This species also occurs on foods, plants and occasionally on optical lenses. *Penicillium purpurogenum* tends to grow in environments with low pH (acidic). A mycotoxin, known as rubratoxin, can be produced when growth occurs on foods. *Penicillium purpurogenum* is not currently recognized as a pathogen, but it has caused a few pulmonary infections in humans and a systemic infection in a dog.

Species Descriptions (Continued)

***Penicillium variabile* (Pvarb2)**
This species is widely distributed in soils and can also be found in seawater, fruit juices, paper and optical lenses. *Penicillium variabile* appears to grow best at slightly acidic pH and does not tolerate high heat for long periods of time. This species produces ochratoxin A (among others) but is not currently known as a pathogen.

***Rhizopus stolonifer* (Rstol)**
This fungus has a worldwide distribution, occurring most densely in soils of warm climates. *Rhizopus stolonifer* is one of the most frequently observed indoor air fungi and commonly grows on foods (e.g. bread) and its spores can germinate on moist paper. It appears that growth is enhanced by slightly alkaline conditions. This species has caused occasional infections, but it is not generally regarded as an important pathogen.

***Scopulariopsis brevicaulis/fusca* (SCbrv)**
S. brevicaulis is the most common species of its genus and occurs worldwide and occurs in soils, floor dust, mattress dust, aquatic environments, compost, seawater, paper mill waste, wood pulp, textiles, paintings and uranium mines. *Scopulariopsis fusca* is also commonly isolated from soil, straw, paper and food. *Scopulariopsis brevicaulis* is regarded as moderately xerophilic, and it can produce toxic by-products of arsenic and mercury, becoming exceptionally dangerous when growing indoors on paints containing arsenic. *Scopulariopsis brevicaulis* is said to produce garlic- or ammonia-like odors when growing indoors. *Scopulariopsis brevicaulis* attacks hairs and keratin, often leading to infections of the toenails and fingernails. However, it can also cause skin, lung and soft tissue infections. *Scopulariopsis fusca* is less frequently pathogenic than *S. brevicaulis*, this species produces infections of the skin and fingernails and toenails.

***Scopulariopsis chartarum* (SCchr)**
Relatively little is known about *Scopulariopsis chartarum*, not to be confused with *Stachybotrys chartarum*. *Scopulariopsis chartarum* was first observed on wallpaper, but has also been found in soils. Growth on maple by this species results in a weakening of the wood. This species does not appear to be a human pathogen, but it has caused a systemic mycosis in a dog.

***Stachybotrys chartarum* (Stac)**
Stachybotrys chartarum is the quintessential black mold found in indoor environments. It is distributed worldwide, primarily found associated on decaying plant material. *Stachybotrys chartarum* possesses a battery of enzymes linked to plant decomposition, making it a potent attacker of all forms of wood, paper and natural fibers (e.g. wool). Hence, it is commonly an indicator of moisture problems in homes and can be found growing on paper, wallpaper, wall board, wood and textiles. *Stachybotrys chartarum* is not a common pathogen, in and of itself, but has garnered particular attention for its role in Sick Building Syndrome, due to its high production of mycotoxins (satratoxin G and H). Long-term exposure to such toxins can induce a myriad of health maladies, including nausea, dermatitis, rhinitis, depression, general malaise, headaches, sore throats, etc. *Stachybotrys chartarum* has also been known to invade lung tissue.

***Trichoderma viride/atroviride/koningii* (Tviri)**
Trichoderma viride and *T. koningii* are cosmopolitan species and have been isolated from almost every environment. Soils, composts and vegetables are common sources of these fungi, and cool and moist environments are preferred. Very little is known about *T. atroviride*. *Trichoderma viride* can grow on linoleum and wallpaper, and is probably more commonly isolated from indoor environments in winter months. As a genus, *Trichoderma* can cause nosocomial (hospital acquired) mycoses from contaminated solutions. *Trichoderma viride* is allergenic and has caused keratitis, peritonitis, pulmonary infections and hematomas.

***Wallemia sebi* (Wsebi)**
This fungus is a very common indoor fungus and is commonly found airborne. It is xerophilic and osmophilic and can be found growing on substrates that would desiccate many other fungi. These substrates include rock salt, bacon, salted foods, jam, jellies, fruits, textiles, rotting paper, and mammals. *W. sebi* can also be found in floor dust, mattress dust, soil and hay. This species is allergenic and is known to colonize human lungs, bones and skin. However, *W. sebi* is not considered a serious pathogen.

Assured Bio Identifier: BW091520-99-1
Sample ID: 12345
Sample Description: Helicopter Pad Entrance

Sample Type: M-TRAP
Collection Volume: 150 L
Reporting Limit: 7 Spores/Cubic Meter

Species Identification	Spores/m^3 of Air Inside	Spores/m^3 of Air Outside	Relative Abundance (%) of Species
Acremonium strictum	ND	ND	0.00
Alternaria alternata	ND	ND	0.00
Anigr*	ND	47	0.00
Aspergillus flavus/oryzae	202	202	46.33
Aspergillus fumigatus, Neosartorya fischeri	ND	ND	0.00
Aspergillus ochraceus/ostianus	ND	ND	0.00
Aspergillus penicillioides	ND	ND	0.00
Aspergillus restrictus/caesillus/conicus	ND	ND	0.00
Aspergillus sclerotiorum	ND	ND	0.00
Aspergillus sydowii	ND	ND	0.00
Aspergillus unguis	ND	ND	0.00
Aspergillus ustus	ND	ND	0.00
Aspergillus versicolor	ND	ND	0.00
Aureobasidium pullulans	12	ND	2.75
Chaetomium globosum	ND	ND	0.00
Cladosporium cladosporioides svar. 1	8	7	1.83
Cladosporium cladosporioides svar. 2	58	26	13.30
Cladosporium herbarum	ND	ND	0.00
Cladosporium sphaerospermum	ND	ND	0.00
Eamst*	99	31	22.71
Epicoccum nigrum	ND	ND	0.00
Muc1*	ND	7	0.00
Paecilomyces variotii	ND	ND	0.00
PenGrp2*	ND	ND	0.00
Penicillium brevicompactum/stoloniferum	50	176	11.47
Penicillium chrysogenum	ND	ND	0.00
Penicillium corylophilum	ND	ND	0.00
Penicillium purpurogenum	ND	ND	0.00
Penicillium variabile	ND	ND	0.00
Pspin2*	ND	ND	0.00
Rhizopus stolonifer	ND	ND	0.00
Scopulariopsis brevicaulis/fusca	ND	ND	0.00
Scopulariopsis chartarum	ND	ND	0.00
Stachybotrys chartarum	ND	ND	0.00
Trichoderma viride/atroviride/koningii	ND	532	0.00
Wallemia sebi	7	ND	1.61
Total Spores:	436	1,028	

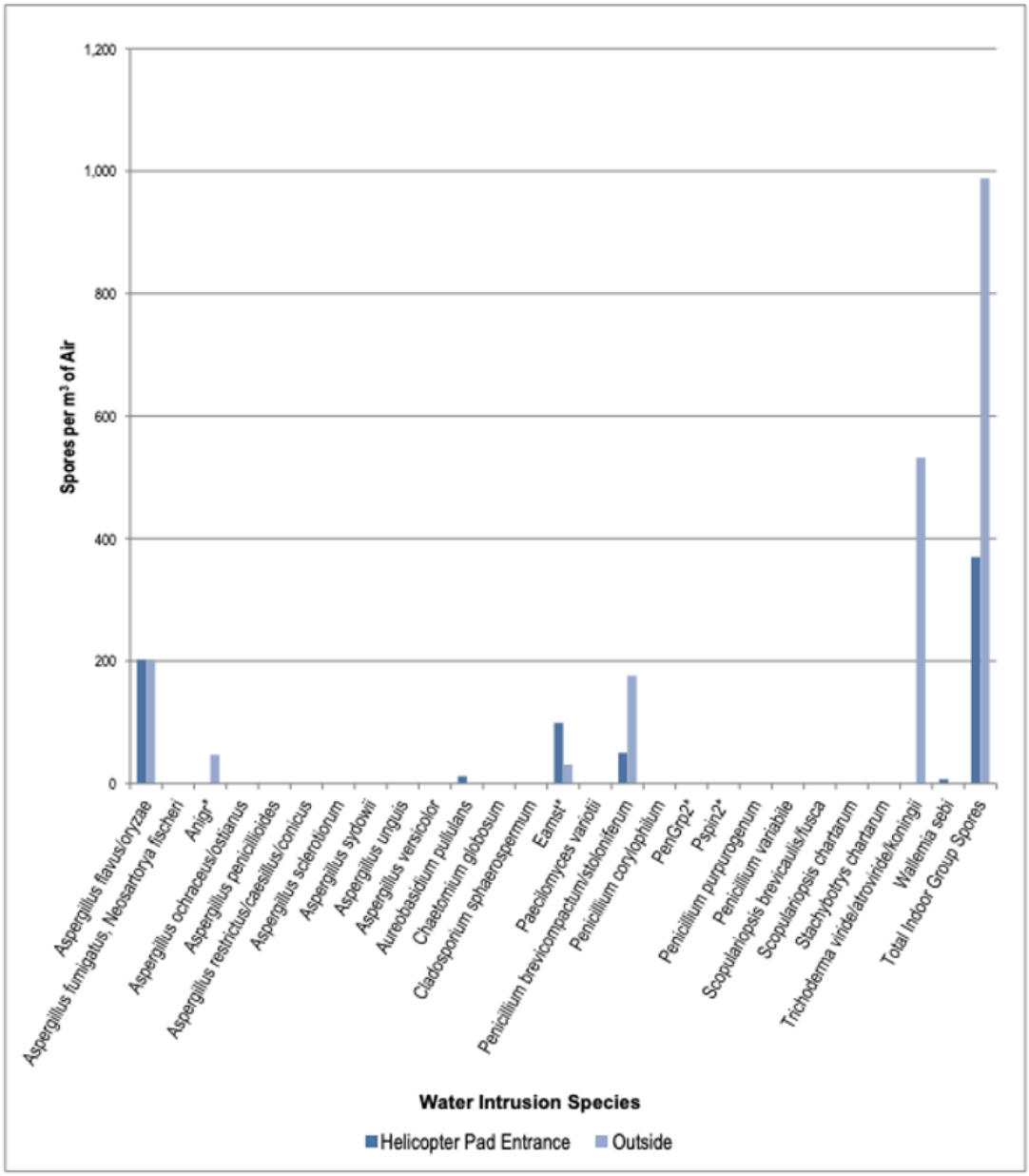
*These assays detect four or more species.

Eamst *Eurotium (Aspergillus) amstelodami/chevalieri/herbariorum/rubrum/repens*
Anigr *Aspergillus niger/awamori/foetidus/phoenicis*
PenGrp2 *Penicillium crustosum/camemberti/commune/echinulatum/solitum*
Pspin2 *Penicillium glabrum/lividum/purpurescens/spinulosum/thomii*
Muc1 *Mucor amphibiorum/circinelloides/hiemalis/indicus/mucedo/racemosus/ramosissimus and Rhizopus azygosporus/homothalicus/microsporus/oligosporus/oryzae*

Results at a Glance: Inside Mold Sample versus Outside Mold Sample

Water Intrusion Molds

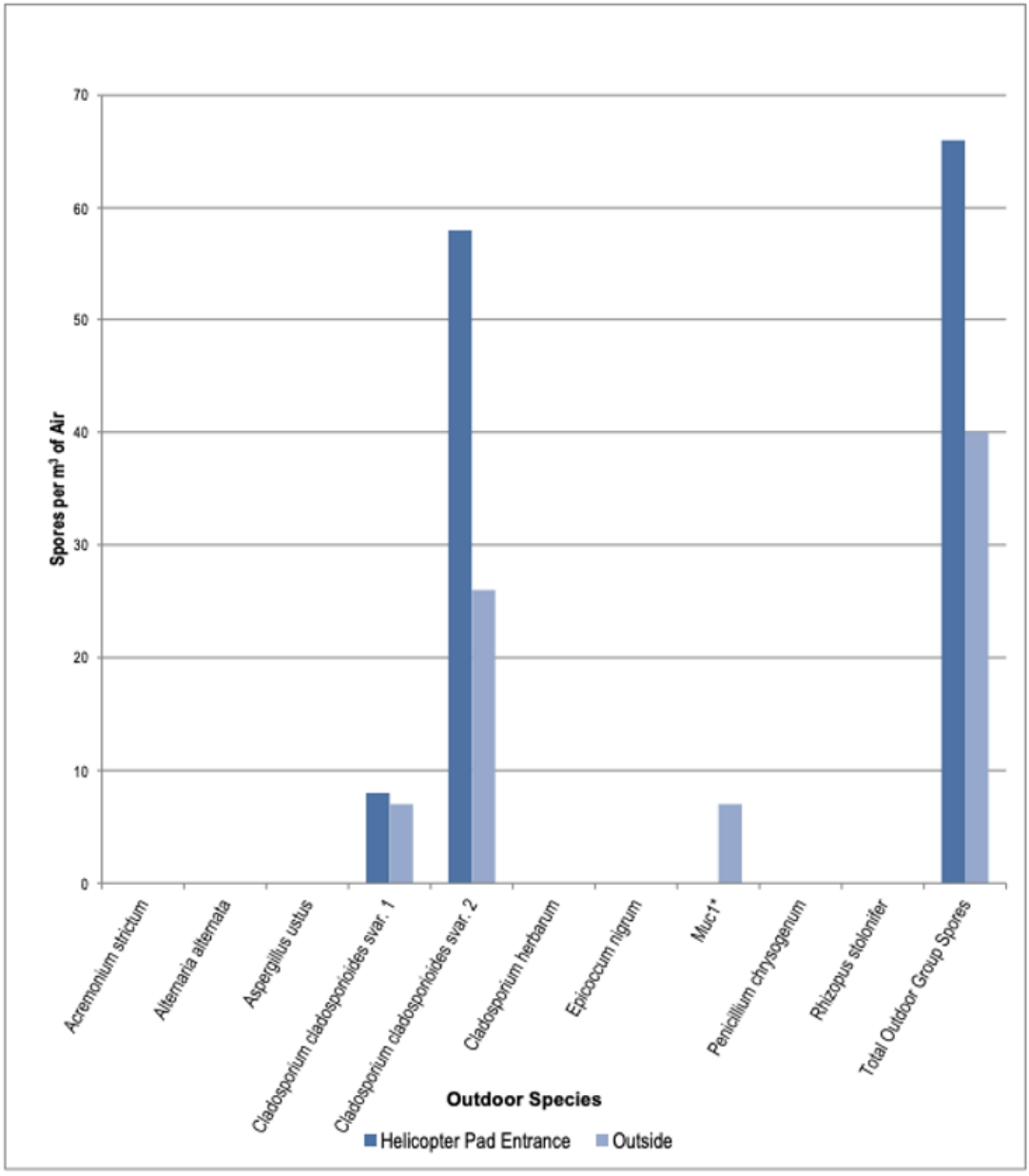
Inside Sample ID:	BW091520-99-1	Helicopter Pad Entrance
Outside Sample ID:	BW091520-99-3	Outside



Results at a Glance: Inside Mold Sample versus Outside Mold Sample

Outside Molds

Inside Sample ID:	BW091520-99-1	Helicopter Pad Entrance
Outside Sample ID:	BW091520-99-3	Outside



Assured Bio Identifier: BW091520-99-2
Sample ID: 23456
Sample Description: Bat Cave

Sample Type: Mtrap
Collection Volume: 150 L
Reporting Limit: 7 Spores/Cubic Meter

Species Identification	Spores/m^3 of Air Inside	Spores/m^3 of Air Outside	Relative Abundance (%) of Detected Species
Acremonium strictum	ND	ND	0.00
Alternaria alternata	ND	ND	0.00
Anigr*	ND	47	0.00
Aspergillus flavus/oryzae	ND	202	0.00
Aspergillus fumigatus, Neosartorya fischeri	ND	ND	0.00
Aspergillus ochraceus/ostianus	ND	ND	0.00
Aspergillus penicillioides	ND	ND	0.00
Aspergillus restrictus/caesillus/conicus	ND	ND	0.00
Aspergillus sclerotiorum	ND	ND	0.00
Aspergillus sydowii	ND	ND	0.00
Aspergillus unguis	ND	ND	0.00
Aspergillus ustus	11	ND	5.95
Aspergillus versicolor	ND	ND	0.00
Aureobasidium pullulans	13	ND	7.03
Chaetomium globosum	ND	ND	0.00
Cladosporium cladosporioides svar. 1	7	7	3.78
Cladosporium cladosporioides svar. 2	131	26	70.81
Cladosporium herbarum	ND	ND	0.00
Cladosporium sphaerospermum	ND	ND	0.00
Eamst*	23	31	12.43
Epicoccum nigrum	ND	ND	0.00
Muc1*	ND	7	0.00
Paecilomyces variotii	ND	ND	0.00
PenGrp2*	ND	ND	0.00
Penicillium brevicompactum/stoloniferum	ND	176	0.00
Penicillium chrysogenum	ND	ND	0.00
Penicillium corylophilum	ND	ND	0.00
Penicillium purpurogenum	ND	ND	0.00
Penicillium variabile	ND	ND	0.00
Pspin2*	ND	ND	0.00
Rhizopus stolonifer	ND	ND	0.00
Scopulariopsis brevicaulis/fusca	ND	ND	0.00
Scopulariopsis chartarum	ND	ND	0.00
Stachybotrys chartarum	ND	ND	0.00
Trichoderma viride/atroviride/koningii	ND	532	0.00
Wallemia sebi	ND	ND	0.00
Total Spores:	185	1,028	

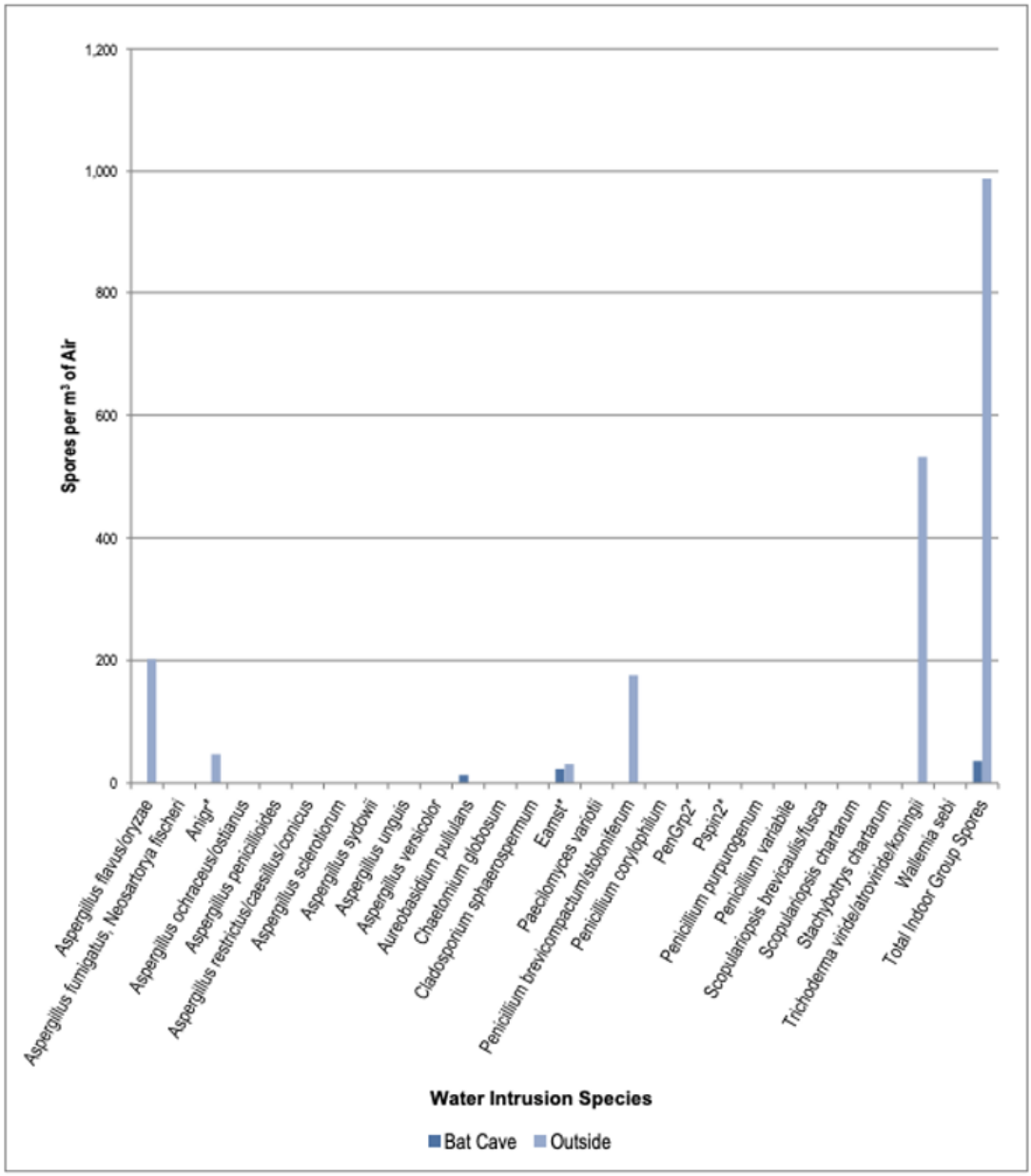
*These assays detect four or more species.

Eamst *Eurotium (Aspergillus) amstelodami/chevalieri/herbariorum/rubrum/repens*
Anigr *Aspergillus niger/awamori/foetidus/phoenicis*
PenGrp2 *Penicillium crustosum/camemberti/commune/echinulatum/solitum*
Pspin2 *Penicillium glabrum/lividum/purpurescens/spinulosum/thomii*
Muc1 *Mucor amphibiorum/circinelloides/hiemalis/indicus/mucedo/racemosus/ramosissimus and Rhizopus azygosporus/homothalicus/microsporus/oligosporus/oryzae*

Results at a Glance: Inside Mold Sample versus Outside Mold Sample

Water Intrusion Molds

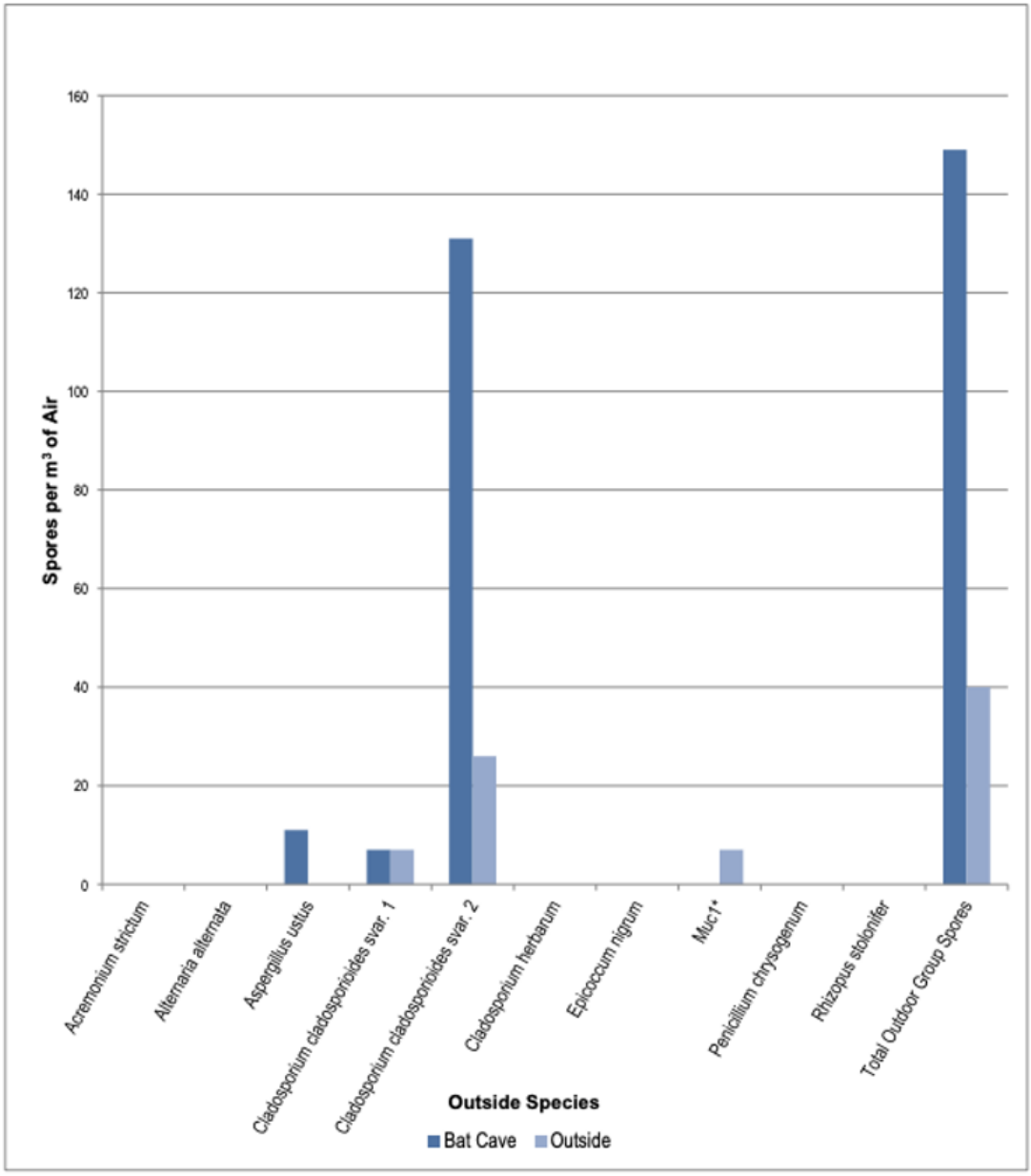
Inside Sample ID:	BW091520-99-2	Bat Cave
Outside Sample ID:	BW091520-99-3	Outside



Results at a Glance: Inside Mold Sample versus Outside Mold Sample

Outside Molds

Inside Sample ID:	BW091520-99-2	Bat Cave
Outside Sample ID:	BW091520-99-3	Outside



Example Report: BIG 2

Sampling Method: Sterile Swab
Number of Samples: 3

Big 2 Panel

PenAsp[1] and Stach[2] Assays

228 Midway Lane, Suite B
Oak Ridge, Tennessee 37830
Toll Free: (866) 547-1727
Local: (865) 813-1700
Fax: (865) 813-1705
Email: info@assuredbio.com
www.assuredbio.com

Inspector:	Assured Bio	**Date Collected:**	1/22/2020
Project Name:	Example Report	**Date Received:**	1/22/2020
Project Number:	1	**Date Reported:**	1/22/2020
Assured Bio Identifier:	AB012220-99	**Analyst(s):**	M. Reed

Selected References

Haugland, R. A., S. J. Vesper and L. J. Wymer. 1999. Quantitative measurement of *Stachybotrys chartarum* conidia using real-time detection of PCR products with the TaqManTM fluorogenic probe system. Molecular and Cellular Probes 13:329-340.

Meklin, T. M., R. A. Haugland, T. Reponen, M. Varma, Z. Lummus, D. Bernstein, L. J. Wymer and S. J. Vesper. 2004. Quantitative PCR analysis of house dust can reveal abnormal mold conditions. Journal of Environmental Monitoring 6:615-620.

Vesper, S. J. 2006. Developing the EPA Relative Moldiness Index© based on mold-specific quantitative PCR. The Synergist April 2006:39-43.

Vesper, S. J., C. McKinstry, C. Yang, R. A. Haugland, C. M. Kercsmar, I. Yike, M. D. Schluchter, H. L. Kirchner, J. Sobolewski, T. M. Alltan and D. G. Dearborn. 2006. Specific molds associated with asthma in water-damaged homes. Journal of Occupational and Environmental Medicine 48:852-858.

Vesper, S., C. McKinstry, P. Ashley, R. Haughland, K. Yeatts, K. Bradhan and E. Svendsen. 2007. Quantitative PCR analysis of molds in the dust from homes of asthmatic children in North Carolina. Journal of Environmental Monitoring 9:826-830.

Accreditation

Assured Bio Labs, LLC is accredited by the American Industrial Hygiene Association Laboratory Accreditation Programs, LLC (AIHA-LAP, LLC; Lab ID # 183867) in the Environmental Microbiology accreditation program for "qPCR - Mold Specific qPCR" Fields of Testing as documented by the Scope of Accreditation Certificate and associated Scope. AIHA-LAP, LLC accreditation complies with the ISO/IEC Standard 17025:2005 requirements, but this does not imply ISO certification or registration."

Limitations

Methods of Analysis

Assured Bio Labs uses the following methods for the MSQPCR analysis: CD 23: Data Reporting for MSQPCR Testing, CD 143: Preparation, Processing, and Analysis of MSQPCR Samples, CD 225: Bead Based DNA Extraction

Notes

[1]The PenAsp assay detects species of the genera *Aspergillus*, *Penicillium*, and *Paecilomyces variotii*.

[2]The Stach assay detects *Stachybotrys chartarum* also commonly referred to as "toxic black mold."

Reporting Limits

Method Detection Limit (MDL): The American Industrial Hygiene Association defines this term in AIHA-LAP, LLC Policy Document – Module 9 as "The minimum concentration of an analyte that, in a given matrix and with a specific method, has a 99 percent probability of being identified, qualitatively or quantitatively measured, and reported to be greater than zero."

Reporting Limit (RL): The American Industrial Hygiene Association defines this term in AIHA-LAP, LLC Policy Document – Module 9 as "The lowest concentration of analyte in a sample that can be reported with a defined, reproducible level of certainty."

Values less than one will be rounded up to one per reported unit.

Method Detection Limits (in Spores)

Stac – 0.1616, PenAsp – 0.2162

Reporting Limit Calculations

Unless otherwise stated in comments, the following equations are used to calculate the reporting limit per sample: Dust RL – MDL/5 mg Swab RL – MDL/1 swab, Unconcentrated Liquid RL – MDL/0.1 ml, Concentrated Liquid RL – MDL/ml filtered, MTrap RL – MDL × (1000/L sampled)

Assured Bio Identifier:	AB012220-99-1	**Sample Condition:**	Intact
Sample ID:	1	**Sample Type:**	Swab
Sample Description:	Upstairs	**Sample Volume:**	1 Swab

Assay	Cells/Swab
PenAsp:	6
Stach:	Below Detectable Limits

Comments: None.

Assured Bio Identifier:	AB012220-99-2	**Sample Condition:**	Intact
Sample ID:	2	**Sample Type:**	Swab
Sample Description:	Downstairs	**Sample Volume:**	1 Swab

Assay	Cells/Swab
PenAsp:	433,049
Stach:	3

Comments: None.

Assured Bio Identifier:	AB012220-99-3	**Sample Condition:**	Intact
Sample ID:	3	**Sample Type:**	Swab
Sample Description:	Basement	**Sample Volume:**	1 Swab

Assay	Cells/Swab
PenAsp:	13,564,374
Stach:	5,158

Comments: None.

Example Report: ERMI

Sampling Method: Dust Collector

Number of Samples: 1

Environmental Relative Moldiness Index (ERMI)

228 Midway Lane, Suite B
Oak Ridge, Tennessee 37830
Toll Free: (866) 547-1727
Local: (865) 813-1700
Fax: (865) 813-1705
Email: info@assuredbio.com
www.assuredbio.com

Inspector:	Certified Industrial Hygienist	**Date Collected:**	1/1/21
Project Name:	Moldy Home	**Date Received:**	1/1/21
Project Number:	123	**Date Reported:**	1/3/21
Assured Bio Identifier:	CIH010121-1	**Analyst(s):**	Dr. Jones

Selected References

Haugland, R. A. and S. J. Vesper. 2002. Method of identifying and quantifying specific fungi and bacteria. US Patent 6,387,652 B1.

Vesper, S. J. 2006. Developing the EPA Relative Moldiness Index© based on mold-specific quantitative PCR. The Synergist April 2006:39-43.

Haugland, R. A., S. J. Vesper and L. J. Wymer. 1999. Quantitative measurement of *Stachybotrys chartarum* conidia using real-time detection of PCR products with the TaqManTM fluorogenic probe system. Molecular and Cellular Probes 13:329-340.

Meklin, T. M., R. A. Haugland, T. Reponen, M. Varma, Z. Lummus, D. Bernstein, L. J. Wymer and S. J. Vesper. 2004. Quantitative PCR analysis of house dust can reveal abnormal mold conditions. Journal of Environmental Monitoring 6:615-620.

Vesper, S. J., C. McKinstry, C. Yang, R. A. Haugland, C. M. Kercsmar, I. Yike, M. D. Schluchter, H. L. Kirchner, J. Sobolewski, T. M. Alltan and D. G. Dearborn. 2006. Specific molds associated with asthma in water-damaged homes. Journal of Occupational and Environmental Medicine 48:852-858.

Accreditation

Assured Bio Labs, LLC is accredited by the American Industrial Hygiene Association Laboratory Accreditation Programs, LLC (AIHA-LAP, LLC; Lab ID # 183867) in the Environmental Microbiology accreditation program for "qPCR - Mold Specific qPCR" Fields of Testing as documented by the Scope of Accreditation Certificate and associated Scope. AIHA-LAP, LLC accreditation complies with the ISO/IEC Standard 17025:2005 requirements, but this does not imply ISO certification or registration."

Disclaimer

ERMI analytical data contained within this report only reflects both the historic and current mold burden within the property tested as of the day the sample was collected. Future mold growth is unknown and can be influenced by water intrusion events such as elevated moisture, condensation, structural or plumbing leaks and/or acts of God (major storm events) that occur subsequent to the ERMI test for which results are documented within this report. If a previous mold remediation was conducted in the property for which these results are being reported, conclusions can only be drawn concerning the current mold burden of the property, not the historic mold burden of the property. The effect of a previous mold remediation or clean-up on the current mold burden of the property is subject to a variety of confounding factors, and drawing conclusions regarding the historic mold burden are cautioned against, unless an ERMI test was conducted following the remediation. In such a case, where an ERMI sample was analyzed following mold remediation, the results of this report should be compared to the post remediation ERMI test data to make inference concerning the historical mold burden of the property. Note: Other forms of post remediation (spore-trap, culturable fungi, etc) are invalid for historic comparison with the ERMI test results contained in this report.

Abbreviations

ND = None Detected

Methods of Analysis

Assured Bio Labs uses the following methods for the MSQPCR analysis: CD 23: Data Reporting for MSQPCR Testing, CD 143: Preparation, Processing, and Analysis of MSQPCR Samples, CD 225: Bead Based DNA Extraction

Notes

The Environmental Relative Moldiness Index (ERMI) is a Quantitative, Real-Time Polymerase Chain Reaction (qPCR) panel of testing for indoor molds that was developed by the United States Environmental Protection Agency (US-EPA). This panel includes 26 mold species and groups of species that are known to thrive in water-damaged homes. This panel also includes 10 species and groups of species of molds that are found in all homes, with or without water damage. Each species and group of species is enumerated from DNA extracted from dust samples taken from both the living and sleeping quarters of homes. Concentrations of each of the 36 molds are used to derive an "ERMI Score" that rates the "moldiness" of each sample against those tested by the US-EPA.

Guidelines to Follow When Interpreting an ERMI SCORE

The Asthma and Allergy Foundation of America has classified the following symptoms for mold allergies: Sneezing, Chronic cough, Runny nose, Nasal congestion, Itchy, watery, and red eyes, Skin rashes and hives, Sinus headaches, Reduced lung capacity and difficulty breathing

Mold-exposure symptoms differ from person to person, depending upon the sensitivities of each individual and their levels of exposure to mold. Persons that are extremely sensitive to mold, or those with suppressed immune systems, could be at higher risk for allergic reactions than those that are less sensitive and have full immune system function. Reaction to mold exposure can be immediate or delayed, depending on the individual and their susceptibility and exposure levels.

The US-EPA has developed a 36-species panel of Mold-Specific Quantitative Polymerase Chain Reaction (MSQPCR) analyses called the Environmental Relative Moldiness Index (ERMI). House dust is used as the medium for this test. Quantities of these species in 1 mg of dust are used to derive an "ERMI Score" that rates the moldiness of a home, based upon scores from approximately 1100 homes tested in the US. Assured Bio Labs recognizes three broad categories of "moldiness" that are of particular importance to occupants of homes. These levels and possible health implications are listed in the ERMI diagnostic chart (see Page 3).

It should be noted that there is no implicit human-health recommendation with an ERMI score. An ERMI score should be used in conjunction with individual mold species quantifications and symptoms of home occupants to arrive at an action decision. An ERMI score is simply a guideline for determining levels of mold exposure for home occupants. As research by the US-EPA and Assured Bio Labs accumulates, interpretations of ERMI scores could change.

We have included the sums of the logs of Group 1 and 2 mold species. These are used for calculating the ERMI score. However, the sum of the logs of Group 2 molds can also be used as a general indicator. This value should be between 7-14 for a home in which mold species have come into equilibrium with outdoor species. Values lower than this usually indicate that the home is new and has not yet equilibrated to the outdoor environment. Values are also commonly low after a remediation event. Values that are high could indicate that cleaning regimes are insufficient, or that a water intrusion event was large enough to cause Group 2 molds to grow in number along with Group 1 molds.

Reporting Limits

Method Detection Limit (MDL): The American Industrial Hygiene Association defines this term in AIHA-LAP, LLC Policy Document – Module 9 as "The minimum concentration of an analyte that, in a given matrix and with a specific method, has a 99 percent probability of being identified, qualitatively or quantitatively measured, and reported to be greater than zero."

Reporting Limit (RL): The American Industrial Hygiene Association defines this term in AIHA-LAP, LLC Policy Document – Module 9 as "The lowest concentration of analyte in a sample that can be reported with a defined, reproducible level of certainty."

Values less than one will be rounded up to one per reported unit. The reporting limit(s) and result(s) are calculated based on the sampling information (i.e. collection volume, area, mass, etc.) provided by the customer as noted on the Chain of Custody. The results apply to the sample(s) as received.

Method Detection Limits (in Spores)

Afumi – 0.6582, Aochr1 – 851.5, Arest – 4.372, Asclr – 0.1649, Aungu – 0.4572, Avers2-2 – 38.19, Apeni2 – 0.3027, Cspha – 0.0328, Eamst – 0.0897, Ppurp – 0.5208, Stac – 0.1615, Aflav – 30.23, Anigr – 0.3142, Asydo3 – 29.95, Apull – 0.0938, Cglob – 0.7785, Pvari2 – 0.0965, Pbrev – 7.549, Pcory – 1.662, PenGrp2 – 5.199, Pspin2 – 13.19, Pvarb2 – 7.758, SCbrv – 0.0284, SCchr – 0.6106, Tviri – 15.21, Wsebi – 7.111, Astrc – 1.346, Aaltr – 42.41, Cclad1 – 0.0403, Cclad2 – 1.049, Cherb – 0.023, Austs2 – 0.0900, Enigr – 0.0051, Muc1 – 0.0244, Pchry – 4.897, Rstol – 0.6515

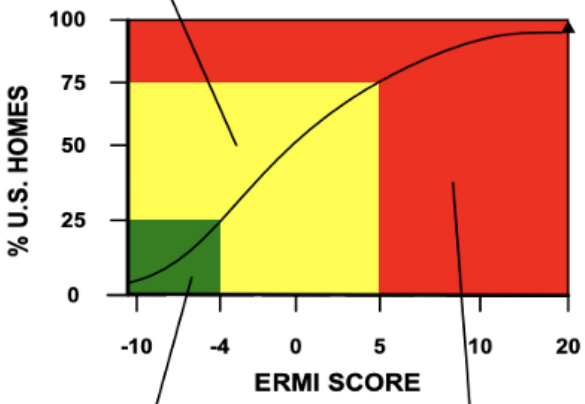
Reporting Limit Calculations

Unless otherwise stated in comments, the following equation is used to calculate the reporting limit per sample: RL = MDL/5 mg.

assuredbio™

ENVIRONMENTAL RELATIVE MOLDINESS INDEX (ERMI)

ERMI = (-4 to 5) MODERATE
Mold-exposure symptoms will vary with the individual. Some individuals exhibit a greater sensitivity to mold than others.



ERMI = (-10 to -4) LOW
These homes hold the lowest ERMI value. Occupants of these homes are least likely to show symptoms of mold exposure. However, mold-exposure symptoms are not impossible.

ERMI = (5 to 20) High
Occupants of these homes are the most likely to show mold-exposure symptoms. However, mold-exposure symptoms are not certain. Some individuals who are least sensitive to mold may remain asymptomatic even when the ERMI score exceeds 5.

assuredbio™

Key to ERMI Assays

Assay name	Target species / group of species
Group 1 Molds	
Afumi	*Aspergillus fumigatus, Neosartorya fischeri*
Aochr1	*Aspergillus ochraceus/ostianus*
Arest	*Aspergillus restrictus/caesillus/conicus*
Asclr	*Aspergillus sclerotiorum*
Aungu	*Aspergillus unguis*
Avers2-2	*Aspergillus versicolor*
Apeni2	*Aspergillus penicillioides*
Cspha	*Cladosporium sphaerospermum*
Eamst	*Eurotium (Aspergillus) amstelodami/chevalieri/herbariorum/rubrum/repens*
Ppurp	*Penicillium purpurogenum*
Stac	*Stachybotrys chartarum*
Aflav	*Aspergillus flavus/oryzae*
Anigr	*Aspergillus niger/awamori/foetidus/phoenicis*
Asydo3	*Aspergillus sydowii*
Apull	*Aureobasidium pullulans*
Cglob	*Chaetomium globosum*
Pvari2	*Paecilomyces variotii*
Pbrev	*Penicillium brevicompactum/stoloniferum*
Pcory	*Penicillium corylophilum*
PenGrp2	*Penicillium crustosum/camemberti/commune/echinulatum/solitum*
Pspin2	*Penicillium glabrum/lividum/purpurescens/spinulosum/thomii*
Pvarb2	*Penicillium variabile*
SCbrv	*Scopulariopsis brevicaulis/fusca*
SCchr	*Scopulariopsis chartarum*
Tviri	*Trichoderma viride/atroviride/koningii*
Wsebi	*Wallemia sebi*
Group 2 Molds	
Astrc	*Acremonium strictum*
Aaltr	*Alternaria alternata*
Cclad1	*Cladosporium cladosporioides* svar. 1
Cclad2	*Cladosporium cladosporioides* svar. 2
Cherb	*Cladosporium herbarum*
Austs2	*Aspergillus ustus*
Enigr	*Epicoccum nigrum*
Muc1	*Mucor amphibiorum/circinelloides/hiemalis/indicus/mucedo/racemosus/ramosissimus* and *Rhizopus azygosporus/homothalicus/microsporus/oligosporus/oryzae*
Pchry	*Penicillium chrysogenum*
Rstol	*Rhizopus stolonifer*

assuredbio™

Assured Bio Identifier:	CIH010121-1	**Sample Condition:**	Intact
Sample ID:	21	**Sample Type:**	Dust
Description:	Master Bedroom and Living Room	**Sample Mass:**	5 mg

Group 1 Mold Species	Assay Name	Spores/mg dust
Aspergillus fumigatus	Afumi	7
Aspergillus ochraceus	Aochr1	ND
Aspergillus restrictus	Arest	ND
Aspergillus sclerotiorum	Asclr	370
Aspergillus unguis	Aungu	ND
Aspergillus versicolor	Avers2-2	ND
Aspergillus penicillioides	Apeni2	ND
Cladosporium sphaerospermum	Cspha	42
Eurotium amstelodami	Eamst	ND
Penicillium purpurogenum	Ppurp	ND
Stachybotrys chartarum	Stac	567
Aspergillus flavus	Aflav	ND
Aspergillus niger	Anigr	ND
Aspergillus sydowii	Asydo3	2,696
Aureobasidium pullulans	Apull	ND
Chaetomium globosum	Cglob	ND
Paecilomyces variotii	Pvari2	1
Penicillium brevicompactum	Pbrev	ND
Penicillium corylophilum	Pcory	ND
Penicillium crustosum	PenGrp2	4,392
Penicillium glabrum	Pspin2	ND
Penicillium variabile	Pvarb2	ND
Scopulariopsis brevicaulis	SCbrv	1,654
Scopulariopsis chartarum	SCchr	ND
Trichoderma viride	Tviri	ND
Wallemia sebi	Wsebi	2

Group 2 Mold Species	Assay Name	Spores/mg dust
Acremonium strictum	Astrc	ND
Alternaria alternata	Aaltr	197
Cladosporium cladosporioides svar. 1	Cclad1	ND
Cladosporium cladosporioides svar. 2	Cclad2	ND
Cladosporium herbarum	Cherb	444
Aspergillus ustus	Austs2	ND
Epicoccum nigrum	Enigr	ND
Mucor amphibiorum	Muc1	1
Penicillium chrysogenum	Pchry	ND
Rhizopus stolonifer	Rstol	ND

Sum of logs of Group 1 species:	18.3
Sum of logs of Group 2 species:	4.9

ERMI Score: **13.4**