Environmental Test Specifications

Mold DNA Panel

Background:

Mold in a house isn't just a problem for people with allergies or asthma. Water leaks in homes provide an ideal environment for mold growth. There are many mold species, black mold or Stachybotrys Chatarum is more dangerous than the most common indoor molds such as Aspergillus and Penicillium. Mold exposure can cause immunosuppression and upper respiratory infections. Long term exposure to mold can cause acute to chronic illnesses.

Test Description:

The Mold DNA test is a quantitative PCR (qPCR) procedure for the detection of ten pathogenic fungal species in environmental dust specimens. It includes six assays that were designed and used by the EPA and four assays that were previously developed by RTL. The qPCR method used in these assays utilizes the hybridization of a species-specific probe to a complimentary DNA strand to amplify and detect fungal DNA. The data generated for each specimen is plotted against a standard curve to calculate the amount of DNA present in the specimen (nanograms of DNA per milliliter of dust in PBS buffer). A process control (*Geotrichum*) is included to verify that the DNA extraction procedure was successful, and PCR positive controls are run with each amplification

References:

- The Bio contaminants and Complexity of Damp Indoor Spaces: More than What Meets the Eyes (Authors: Thrasher JD and Crawley S). *Toxicology and Industrial Health*. 2009.
-Mycotoxin Detection in Human Samples from Patients Exposed to Environmental Molds. Hooper, D.G., Bolton, V.E., Guilford, F.T. and D.C. Straus. Int. J. Mol. Sci. 2009, 10, 1465-1475
-Indoor Environmental quality – Dampness and mold in the buildings https://www.cdc.gov/niosh/topics/indoorenv/mold.html.

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Assay Method: Quantitative PCR (qPCR)

Mold DNA Targets:

Aspergillus fumigatus	*Aspergillus ochraceus*	*Candida auris*
Aspergillus flavus	*Aspergillus terreus*	*Chaetomium globosum*
Aspergillus niger	*Aspergillus versicolor*	*Fusarium solani*
		Stachybotrys chartarum

PCR Amplification Efficiency

Amplification efficiency was evaluated by obtaining concentrated DNA from an independent vendor for all assays and serially diluted ten-fold to produce dilutions of 1/10, 1/100, 1/1000, and 1/10000. The dilutions for each assay were amplified in triplicate to obtain amplification efficiency. All assays demonstrated a high amplification efficiency. Amplification efficiency was calculated using the following equation:

Efficiency = -1+10^(-1/slope) *100%

Assay	*A. flavus*	*A. fumigatus*	*A. niger*	*A. ochraceus*	*A. terreus*
Efficiency	77.7%	86.0%	107.6%	76.4%	96.4%

Assay	*A. versicolor*	*C. auris*	*C. globosum*	*F. solani*	*S. chartarum*
Efficiency	67.3%	93.7%	105.1%	97.2%	98.7%

Precision/Reproducibility

Assay precision was determined by testing twenty replicates of positive controls over several days and between multiple technologists. Inter-run cycle threshold values display high precision with less than 5% CV in all assays.

Linearity

All qPCR assays are highly linear ($R^2 > 0.97$) over several orders of magnitude.

Limit of Detection (LOD)

Assay LOD was determined for all assays by obtaining concentrated DNA and diluting the DNA down to 1000 nanograms per mL of dust in PBS buffer. DNA was further serially diluted ten-fold down to 0.001 ng/mL. LOD samples were processed in triplicate. The amount of DNA (ng/mL) detected in >95% of replicates is presented below:

Assay	*A. flavus*	*A. fumigatus*	*A. niger*	*A. ochraceus*	*A. terreus*
>95% Detection	0.657	0.012	0.015	1.810	1.393

Assay	*A. versicolor*	*C. auris*	*C. globosum*	*F. solani*	*S. chartarum*
>95% Detection	2.625	0.065	0.038	0.314	0.292

Specificity

Assay Specificity was determined by obtaining purified DNA and processing with each assay. All prepared samples were run with all assays in triplicate. All assays show 100% specificity for their intended target.

Accreditation

RealTime Laboratories, Inc. is a CAP (#7210193) and CLIA (#45D1051736) accredited testing laboratory.