Environmental Test Specifications

EMMA 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 EMMA test is a quantitative PCR (qPCR) procedure for the detection of ten pathogenic fungal species in environmental dust specimens. EMMA 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 Biocontaminants 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.

Environmental Test Specifications

Background

Mycotoxins are small molecular weight toxic molecules produced by various species of mold, some of which inhabit water damaged homes or buildings. Many clinical symptoms and disease states have been associated with human exposure to mycotoxins. The RealTime Lab Mycotoxin Panel detects 15 different mycotoxins, as follows: Trichothecenes (Satratoxin G and H, Isosatratoxin F, Roridin A, E, H, and L-2, and Verrucarin A and J.), Ochratoxins (Ochratoxin A), Aflatoxins (Aflatoxin B1, B2, G1, and G2), and Gliotoxin (bis (methyl) gliotoxin)

Mycotoxins are particularly important because they are known to be highly toxic and are produced by species such as Stachybotrys ("black mold"), shown to be present in mold contaminated buildings.

Testing is done using competitive ELISA, a very sensitive and specific method for detection using antibodies prepared against the Mycotoxins.

References:

- 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
- Chronic Illness Associated with Mold and Mycotoxins: Is Naso-Sinus Fungal Biofilm the Culprit? Brewer, J.H., Thrasher, J.D., and D. Hooper. Toxins. 2014. Jan; 6(1):66-80
- Intranasal Antifungal Therapy in Patients with Chronic Illness Associated with Mold and Mycotoxins: An Observational Analysis. Brewer, J.H., Hooper, D., and S. Muralidhar. Global J. of Med. Res. 2015. 15(1). 29-33
- Trichothecenes: From Simple to Complex Mycotoxins. McCormick, S.P., Stanley, A.M., Stover, N.A., and N.J. Alexander. Toxins. 2011. 3, 802-814
- Enzyme Immunoassay for the Macrocyclic Tricothecene Roridin A: Production, Properties and use of Rabbit Antibodies. Martlbauer, E.,

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EMMA Mycotoxin Panel

Assay Method: ELISA

Accuracy

Assay accuracy was evaluated by obtaining concentrated mycotoxin from an independent vendor, and the RTL Mycotoxin Panel was used to measure the concentration of each mycotoxin present within a range of dilutions specific for each assay. Measurements must be accurate within 20% of the expected value for samples measured in the assay specific ranges.

Assays	Tested Concentration Range (PPB)	Percent Error
Trichothecene	0.01 to 1.0	≤ 20.0
Ochratoxin	0.5 to 10.0	≤ 20.0
Aflatoxin	1.0 to 8.0	≤ 20.0
Gliotoxin	0.3 to 10.0	≤ 20.0

Precision/Reproducibility

Assay precision was determined by spiking a negative urine sample with a known amount of mycotoxin and testing ten replicates for each assay. Measurements must have a Coefficient of Variation (CV) of ≤ 20%.

Assays	Tested Concentration Range (PPB)	Coefficient of Variation (%CV)
Trichothecene	0.01 to 1.0	≤ 20.0
Ochratoxin	0.5 to 10.0	≤ 20.0
Aflatoxin	1.0 to 8.0	≤ 20.0
Gliotoxin	0.3 to 10.0	≤ 20.0

Linearity

The RTL Mycotoxin Assays are highly linear ($R^2 > 0.95$) over several orders of magnitude. The reportable ranges for the assays are as follows:

Assays	Present if ≥	Equivocal if between
Trichothecene	0.03 ppb	0.02-0.03 ppb
Ochratoxin	2.0 ppb	1.8-2.0 ppb
Aflatoxin	1.0 ppb	0.8-1.0 ppb
Gliotoxin	1.0 ppb	0.5-1.0 ppb

Sensitivity

The analytical limit of detection for the mycotoxin assays are as follows: Trichothecene is 0.01 ppb, Ochratoxin is 0.5 ppb, Aflatoxin is 1.0 ppb, and Gliotoxin is 0.3 ppb.

Specificity

The RTL Mycotoxin Panel is specific for the detection and measurement of 15 specific mycotoxins. Each assay is specific for the mycotoxins specified and do not cross react with any other mycotoxins in the same sample thus not yielding a false positive result.

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