December 21, 2007

NIOSH Docket Office
ATTN: Docket Number NIOSH-091
Robert A. Taft Laboratories
4676 Columbia Parkway, M/S C-34
Cincinnati, OH 45226
Attn: Diane Miller

http://www.cdc.gov/niosh/review/public/77-173

Re: Comment of Roche Diagnostics on the NIOSH Sampling Strategies Manual

Dear Ms. Miller:

On behalf of Roche Diagnostics, we appreciate this opportunity to submit comments on the NIOSH Sampling Strategies Manual. We particularly appreciate the opportunity to comment on the Manual, which, as NIOSH acknowledges, has been last revised several decades ago. Given the Manual's prominence in the industrial hygiene community, we commend the Agency on its initiative to reopen the document and solicit comments from the industrial hygiene community. As set forth in more detail below, we urge NIOSH to include within the manual sampling strategies for indoor air quality, and specifically for mold.

For the past ten years, scientists at the U.S. Environmental Protection Agency (EPA) have worked to develop a highly sophisticated DNA test to identify and quantify molds in the indoor environment. This assay, upon which the Environmental Relative Moldiness Index (ERMI) is based, is a rapid, highly accurate DNA-based, dust detection method that uses quantitative polymerase chain reaction (qPCR) technology to detect the presence of molds commonly associated with water intrusion and respiratory disease. In 2006, EPA tested a representative sample of more than 1,100 homes across the country as part of the U.S. Department of Housing and Urban Development's (HUD's) 2006 American Healthy Homes Survey. The results of this testing formed the backbone of the ERMI Index.[1]

In addition, recently dust/qPCR sampling method and ERMI assay was selected by the National Institute of Child Health and Human Development (NICHD) to be utilized in the National Children's Study (NCS), in lieu of traditional culture methods, because of its quantitative nature, rapid sample turn around (24-48 hours) and simplicity of sample collection. No other analytical method provides the specificity and sensitivity to produce the consistent, standardized results necessary to explore the causal link between mold exposure (*Aspergillus sp.*, *Alternaria sp.*, etc.) and current and future health risks.

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[1] Stephen Vesper, et al, *Development of an Environmental Relative Moldiness Index for US Homes*, 49 J. of Environmental Med. 829 (2007).

The dust/qPCR methodology and ERMI assay is a breakthrough in standardizing mold testing. Prior to the dust/qPCR method, mold technologies suffered from massive variability in both sampling and analysis. Given the dust/qPCR sampling and the ERMI analytic breakthrough, which for the first time enables precise, accurate and robust mold test, we believe NIOSH can include within the Manual provisions that speak to both mold sampling strategies and methodologies. The assay is commercially available, and can be ordered through most of the larger environmental testing laboratories, and has been employed in many epidemiological studies published in the peer-reviewed literature. As demonstrated below, and as is borne out by the literature, traditional mold air sampling and sample culturing are demonstrably inaccurate and imprecise (both generally, and as NIOSH defines those terms). Thus, we believe NIOSH has an important opportunity to address new mold sampling strategies, and to clarify that only dust/qPCR testing will produce reliable and verifiable testing results. Thus, we strongly urge you to include specific references to dust/qPCR sampling and ERMI analysis in the revised Sampling Strategies Manual.

A. Sample Collection for dust/qPCR testing is more efficient and reliable than other methods of mold detection.

Prior to the development of the dust/qPCR test, mold testing was commonly conducted using spore traps or culture-based methods. These methods have significant limitations that make them inappropriate for mold testing due to their subjective nature of analysis and other deficiencies described below. In contrast, dust/qPCR analysis relies on a standardized and largely automated process that eliminates the subjectivity and risk of human error common to these other methods.

i. Spore Trap Analysis

Spore trap analysis is a non-culture based diagnostic tool that provides an indication of airborne mold spores present in the indoor environment at the moment sampling is taking place. Spore traps are essentially a grab sample or snapshot of the indoor air at a single moment in time. Spores are released on diurnal schedules that are specific to the species of mold. Also fluctuations of temperature, humidity and various disturbances, such as frequency of cleaning or a change in seasons can induce or suppress spore release. In order for a spore trap to determine indoor mold contamination with any level of accuracy, 24-hour monitoring, in multiple rooms over a period of days would be required. The dust/qPCR method however, requires only a single composite sample of dust from two designated areas in order for an analysis to be rendered. Moreover, the spore trap method cannot provide species identification, as does the dust/qPCR method. The spore trap method only identifies molds to genus or broad categorical groups.

In addition, spore trap analysis is not standardized, as there currently is no standardized method for sampling or analysis of spore traps. Direct microscopy is used to analyze spore trap samples, which requires visual identification and tallying of spores by lab technicians who often possess varying skills, experience, and training. The results also tend to vary based upon the laboratory used, and even between analysts in the same laboratory.

ii. Culture-Based Mold Detection

Mold culturing methods provide some advantages over spore trap for identifying mold species, however, culturing fungi from air is also problematic. To be more specific, not all molds will grow

on the same growth medium or at the same rate, and since culture based species identification is an extremely complex process, a qualified Ph.D. Mycologist is required to ensure an accurate identification. Furthermore, culturing requires multiple isolation and media transfer steps. The entire process is time consuming and typically takes 15-20 days to report results. By contrast, with the dust/qPCR method, technicians can extract and analyze the DNA for an ERMI score in 2-3 hours.

In addition, the dust/qPCR method detects DNA from both non-viable and viable mold spores. Non-viable mold spores are still allergenic and possibly contain a variety of mycotoxins. In addition, culture-based testing cannot be relied on to provide quantitative results, with recent research indicating this method is also not standardized and significantly underestimates mold concentrations when compared to dust/qPCR results. Hence, dust/qPCR sampling strategies provide a superior alternative without the limitations that exist in spore trap and culture-based mold detection.

B. Due to its enhanced reliability, accuracy, and precision, dust/qPCR testing is significantly more efficient than other methods of mold detection.

Dust/qPCR sampling strategy renders not only a more reliable, accurate, and precise measure of 26 "indicator" species of abnormal mold found in buildings with water damage, but also provides a more efficient and, as such, a more cost-effective strategy than that of other mold detection methods. Dust/qPCR can detect these indicator species regardless of regional, seasonal, and occupant-specific variability. Further, as dust/qPCR is a composite sample, sampling can be undertaken in multiple locations, rather than having to run multiple tests in each area of concern. In other words, since dust/qPCR sampling and analysis requires only a single sample of dust, efficiency is gained and the costs associated with repeated air sampling in multiple locations in a building or home can be avoided.

C. Sampling Strategies and the Collection of Samples

One of the additional benefits of dust/qPCR sampling, beyond the accurate, precise, and quantitative measure of the mold that is present, is the simplicity of the apparatus used to collect the sample. All that is needed is a simple dust cartridge that can be fitted onto the end of a standard vacuum. Sampling should be collected over an appropriate time (approximately 5 minutes) by running the vacuum continuously over a 2 foot square area. Like other sampling, sampling should protect against potential contamination of the specimen, and should also ensure that the quantity of dust collected is sufficient to support a valid analysis (known because the vacuum cartridge through an observation window itself provides the ability to verify, in real-time, that the requisite quantity of dust has been collected).

Conclusion

We appreciate the significant effort invested by NIOSH in updating the Sampling Strategies Manual, and hope our comments above are valuable in the effort. We urge NIOSH to include dust/qPCR method in the revised Manual as the superior mold evaluation strategy. Of course, we welcome any questions you may have, and are available at dfarber@pattonboggs.com or

oliver.strobel@roche.com at your convenience.

Thank you.

Sincerely,

David J. Farber

David Farber

cc: W. Gregory Lotz, Ph.D.
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