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REVIEW ARTICLE

Traditional mould analysis compared to a DNA-based method of mould analysis

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Abstract
Traditional environmental mould analysis is based on microscopic observations and counting of mould structures collected from the air on a sticky surface or culturing of moulds on growth media for identification and quantification. These approaches have significant limitations. A DNA-based method of mould analysis called mould specific quantitative PCR (MSQPCR) was created for more than 100 moulds. Based on a national sampling and analysis by MSQPCR of dust in US homes, a scale for comparing the mould burden in homes was created called the Environmental Relative Mouldiness Index (ERMI).

Keywords: *Mould; ERMI; MSQPCR; home; dust*

Introduction

The analysis of mould populations in homes has historically been completed by some type of capturing or collection of a sample, most often from the air but sometimes from dust or building material (e.g., dry wall), and the microscopic enumeration, either directly by observing the spores/cells/fragments or by culturing from the sample collected. The Department of Housing and Urban Development (HUD) in its Report to Congress described the resulting situation succinctly (HUD 2005).

"Standard approaches to mould testing include: (1) viable count methods that involve collecting spores in air and dust samples or through direct contact with the mould, then culturing the spores on nutrient media and counting the number of colonies that grow and classifying them by species: and (2) spore counts that involve counting the number of mould spores in air or dust samples and, if possible, identifying individual species or groups. These techniques are time consuming and require considerable technical expertise. Another problem is the difficulty in interpreting test results, since mould spores are ubiquitous and there is no consensus among experts regarding what constitutes acceptable indoor spore concentrations in indoor air or house dust, or which species are most problematic."

HUD also noted (HUD 2005): "Yet even now there are situations where reliable test methods are needed, including the identification of hidden mould problems and …to better define mould-related hazards based on significant association with adverse health effects in residents." The World Health Organization Report (WHO 2009) described these technologies as having "serious flaws." One of the major recommendations espoused by the Institutes of Medicine report (IOM 2004) regarding mould, moisture and health was the need for the development of a molecular-based method of mould analysis.

This review will summarize traditional approaches to mould sampling, identification/ quantification, and data interpretation and their limitations. The development of an alternative DNA-based analysis, mould specific quantitative PCR (MSQPCR) (Haugland and Vesper, 2002) will be discussed and illustrated with examples. Applying MSQPCR to understanding mould populations in homes across the United States resulted in the creation of the Environmental Relative Mouldiness Index (ERMI) scale (Vesper et al., 2007b). To illustrate the use of the ERMI scale, four epidemiological studies of childhood asthma in which the ERMI scale was used to characterize the mould burden will be summarized.

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Sampling methods

Methods for sampling moulds can range from "not sampling" to the very simple tape or bulk sample to vacuum sampling. Depending on the question that is being addressed, each sampling method has its applications and limitations (Niemeier et al., 2006).

If there has been flooding in a home resulting in obvious mould growth, it is unlikely that any mould sampling or analysis is required. Under these circumstances, major remediation may be in order. However, this situation represents only a small portion of the water-damaged homes. A tape or bulk sample for microscopic observation should be sufficient to make a mould versus non-mould determination. This kind of sample can be helpful when an initial visual assessment is being made of the potentially problematic areas in a home.

If a surgeon wants to know if a hospital operating room contains *Aspergillus fumigatus* cells, then air samples might be sufficient. These air samples should be of a length and volume to provide the assurance that the surgeon and hospital administrators need.

However, to estimate a child's exposure to mould in a home over a number of years, dust sampling is the only practical approach. In spite of the fact that the length of the dust accumulation in any particular home is not known and that the respirable fraction of the dust is not known, the results described below will indicate that home dust may represent a viable option in understanding childhood mould exposures associated with asthma development and/or exacerbation.

No sampling

In 2007, ASTM International promulgated the standard D 7297 "Practice for Evaluating Residential Indoor Air Quality Concerns" (ASTM 2007). The first phase of this Standard is based on a "walk-through" examination of the home. The use of a "walk-through" is frequently the initial method in a mould investigation. In some cases, the mould growth is easily found because it is covering whole walls and ceilings. In these cases, there is generally no need for mould sampling. This is especially the case when the source of the water has been identified. The limitations of this approach are the differences in effort that each inspector makes in finding the mould, the varying degree of human olfactory effectiveness in detecting mould and lack of standardization in quantifying what is found.

Swab, sticky tape, or bulk sampling

Swab, sticky tape, and bulk samples (usually pieces of wood or dry wall) are useful for differentiating mould from non-mould. Most of the time, the characteristic mycelial or mould spore appearance under the microscope can be easily determined by a trained mycologist from such samples. But, in general, it is not possible to identify the mould species based on this kind of sample without cultivation on growth media. The swab or sticky tape sample is not a quantitative sample and does not provide information about other moulds which are not directly on the sampled spot or piece of building material.

Air sampling

Historically, short air samples using various types of impact collectors (Air-O-Cell Cassette™, Micro5 Cassettes™, and so on) have been the dominant method of mould sampling in the environmental field. The particles in the air are often captured on a sticky surface and "read" by microscopic observation and counting. Each collection device has its own advantages and disadvantages, with different inlet dimensions and shapes affecting the ability to collect particles of different sizes and to deposit the particles uniformly (Grinshpun et al., 2007). The efficiency of air sampling is also affected by relative humidity which can change the aerodynamic size of the particles, either expansion under wet conditions or contraction in low humidity (Tucker, 2006).

As an alternative to the use of sticky collection surfaces, impactors like the Andersen Sampler™ or BioCassette™ collect the particles onto growth media. For example, the Andersen sampler separates particles by their aerodynamic size onto a growth medium. The Petri dishes are recovered and incubated for various times and at various temperatures. Only short sampling times (5–10 minutes) are possible, otherwise the plates would be overgrown (Johnson et al., 2008). In addition, not all moulds grow at the same rate or on the same media which causes a selection for certain moulds that are easily cultured.

Newer air sampling devices like the Biosampler™, RCS High Flow Sampler™, Cyclone Bioaerosol Sampler™, and so on allow for longer air sampling times, e.g., hours instead of minutes (An et al., 2004; Macher et al., 2008). Each has advantages and disadvantages depending on the target but, no matter what the device; the analysis is still limited by the same problems of identifying and counting moulds that were described above. Although these collectors can sample for longer periods, none can provide an estimate of mould exposure over a period of months to years.

Dust sampling

Many different configurations and instruments have been used to collect dust samples including various types of vacuum cleaners, wiping cloths, and so on. HUD and EPA (Vesper et al., 2007b) for the 2006 American Healthy

Homes Survey (AHHS) utilized the MiTest™ dust sampler (Indoor Biotechnologies, Charlottesville, VA) which is very easy to use and inexpensive. The rooms sampled in the home were standardized to the bedroom and living room because every home has a bedroom and living room but many do not have attics or basements, for example. The areas sampled were 2 m² in each room for 5 minutes. In some circumstances, the standard sample cannot be obtained and in those cases the vacuum cleaner bag dust was substituted (Vesper et al., 2009b).

Recent home alterations (remodeling, new carpets, and so on) which affect dust deposition and retention, can affect the interpretation of the standard dust sample. It is therefore important to inquire about recent changes in the home. If there has been a significant change in the home, then alternative sources of undisturbed dust should be sought. These sources could include tops of doorways, window sills, bookshelves, and so on.

Major issues in environmental/commercial mould analysis

The science of taxonomy is very exacting and requires significant training and expertise. The identification of moulds by taxonomists requires careful measurement and painstaking observations at different stages of mould development, often requiring growth on multiple media. For routine applications in environmental and commercial studies of mould populations, these approaches present significant challenges, especially where standardization and reproducibility are desired.

The analysis of environmental samples is often performed by technicians with variable training and under great time pressure (Brandys, 2007). Environmental samples might contain tens to hundreds of species and anywhere from one to millions of mould cells. Mould cells may be hidden from microscopic observation in a background of other particles from the air. Sometimes these other particles can look like mould cells or structures. Therefore it should not be surprising that comparative analyses of the same samples by different laboratories produced highly variable results (Godish and Godish, 2006; Brandys, 2007). The interpretation of the results from this kind of mould analysis has never been standardized (HUD, 2005).

Identification and quantification

Traditional mould quantification was based on the enumeration of cells captured on a sticky surface and counted under a microscope or by culturing moulds from the sample on various media. Most moulds cannot be identified simply by looking at the spores. Because of this limitation, most commercial mould analyses only describe the moulds to the genus level. And in the cases of *Aspergillus* and *Penicillium* cells, these two genera cannot be distinguished by microscopic observation alone. For that reason, most commercial labs simply combine these genera together as the mega-category "Asp/Pen." In other cases, the mould cells or structures are placed in category "unidentified." In addition, commercial labs typically allow the technician only 6–8 minutes to count a slide (Brandys 2007). These limitations have now been documented.

Brandys (2007) sent the same set of four slides to seven AIHA certified laboratories. Not only were there significant variation in the counts produced by the different labs but even the identification at the genus level was inconsistent. He summarized the results of the overall study by noting that, "there is so much variance in this data that little statistically useful information can be gained" (Brandys, 2007).

Another study of 10 commercial, AIHA credited laboratories evaluated the variability of total spore/particle counts and culturable mould sample concentrations (Godish and Godish, 2006). The authors summarized their findings by noting that "as a general rule, total mould spore/particle concentrations reported by commercial laboratories may not be reliable indicators of total airborne mould spore/particle levels and thus potential human exposures."

The alternative to counting is culturing the moulds from the sample on various growth media. Many mould colonies look very similar on one medium and not another and specific media will select for different moulds. Technicians vary widely in their experience in identifying mould colonies and rarely have time to confirm their observations.

Clumps of cells will produce a single colony and result in an underestimate of the concentration. Dead cells go undetected even though they remain potentially toxic and allergenic. In addition, there are many human steps in the process of culturing moulds, e.g., making dilutions, where human errors can be amplified. For all of these reasons, population estimates based on culturing are usually underestimates (Meklin et al., 2004).

Interpretation of the mould analysis data

Historically, the commercial laboratories performing the traditional mould analysis provided little to no interpretation of the data generated from the samples analyzed. Their final report was merely a set of numbers. Even after many years of traditional mould analysis, there is no accepted method for interpreting such mould data (HUD, 2005). This weakness in interpretation has led to great confusion and the creation of many "professional judgment" methods of mould data interpretation.

One procedure used by some to estimate if a building has an abnormal mould condition is the comparison of indoor and outdoor mould concentrations in air samples (Gots et al., 2003). Often the comparison is based on the ratio of the total number of spores, or totals for a few genera, quantified by either spore counting or culturing on one or two media. However, in a comparison study of indoor and outdoor moulds at the species level (as opposed to the genus or higher taxonomic level), Meklin et al. (2007) found that there was little correlation between the moulds found indoors versus outdoors.

The lack of adequate identification and quantification of the moulds has produced a spectrum of "professional judgment" methods of interpreting mould population data, usually based on an individual's experience. Sometimes a "rule-of-thumb" is used, e.g., two-times higher mould counts indoors than outdoors means there may be an indoor mould problem (Byggmeister Associates, 2008). Johnson et al. (2008) recently summarized the end result of this situation by noting that: "professional judgment in the evaluation of airborne mould sampling data leads to inconsistent conclusions regarding the presence of an indoor mould source." For these reasons a standardized approach to mould identification and quantification was needed to produce a more consistent, less subjective interpretation of mould population data.

Molecular approach to mould identification and quantification

To create a DNA-based mould analysis, it was necessary to discover a DNA sequence that is unique to each species and yet stable enough to encompass all members of that particular species. Specific regions of the microbial genome are fairly "stable," e.g., the internal transcribed spacer (ITS) sequences, some mitochondrial introns, and so on (Santamaria et al., 2009). These stable regions are now being used in the identification of microorganisms (Blaxter, 2004). If a DNA-based method for the identification of moulds was created, then the polymerase chain reaction (PCR) could be used for performing quantification (or semi-quantification).

The development of PCR in the early 1980s provided a method of very precisely reproducing a segment of DNA in a controlled manner. One of the many applications of this technique was the amplification of DNA sequences unique to species of moulds (Haugland and Heckman, 1998). However the quantification of the target species was limited because only the end product of the amplifications process could be measured which is only semi-quantitative. What was lacking was a method to monitor the entire amplification process.

This problem was solved with the development of real-time or quantitative PCR (QPCR) (Heid et al., 1996). With QPCR, a "probe" with fluorescent dyes was incorporated into the amplification process so that each amplification step could be monitored by an instrument called a Sequence Detector. With this development, a highly accurate method of quantifying the targeted sequences became available. Today, QPCR technology is widely used with hundreds of peer-reviewed papers supporting its conclusions. At the EPA, researchers decided to apply this technology to mould identification and quantification (Haugland et al., 2002).

The moulds targeted for QPCR assay development were based on the scientific literature regarding moulds found indoors and genera in which some semblance of genetic understanding of the species already existed. EPA scientists designed and tested probes and primers (called an assay) for over 100 moulds (http://www.epa.gov/microbes/moldtech.htm) and designated the resulting technology as mould specific quantitative PCR (MSQPCR).

MSQPCR analysis can be performed on dust or air samples. Dust is collected, usually with a MiTest™ sampler, and sieved through a 300 μm pore size nylon mesh screen. Then 5 mg of dust is placed in a 2 ml tube called the "bead-beating tube (Haugland et al., 2002). Air samples are collected on polycarbonate or Teflon™ filters (0.8 μm pore size). The entire filter is removed from the sampling holder and placed in a 2 ml "bead-beating" tube. Each dust or air sample tube is spiked with 1×10^6 conidia of *Geotrichum candidum* as an external reference, and then extracted by a rapid mechanical bead-milling method at 5,000 rpm for 1 min. (Haugland et al., 2002) and DNA purified with the use of a commercial kit.

The MSQPCR assays are species specific, since they are based on the "Type Strain" (i.e., the strain deposited in a culture collection when the mould was first named and described) for each of the species, if that Type Strain still exists (Haugland et al., 2004). In a few cases, e.g., *Stachybotrys chartarum*, the Type Strain has been lost and consensus sequences were built (Haugland et al., 1999). The results for the assays are compared to standard curves generated from spore suspensions of a known concentration of each target mould. MSQPCR assays provide quantitative results that are linear over at least six orders of magnitude of cell concentrations (Haugland et al., 2004). Enumeration results have a 95% confidence range of one-half log (Haugland et al., 2004). In most cases, these assays are sensitive to a single spore or a few spores per sample and detection of any other species by the assay would need at least 1,000-fold more spores than for the target mould (Haugland et al., 2004).

In some cases, formerly separate "species" were found to be genetically identical and therefore these

species were measured in a single assay, e.g., the assay *Penicillium* assay 2 is made-up of several previously separated *Penicillium* species (Haugland et al., 2004). In other cases, historically single species, which are actually genetically different, were subdivided into sub-species. For example, *Cladosporium cladosporioides* is actually two different genetic sub-species which are identified and quantified using two separate MSQPCR assays (Haugland and Vesper, 2002).

Results from MSQPCR were compared to traditional culture-based methods for the identification and quantification of moulds. The same set of samples was evaluated by an "expert" and by MSQPCR (Meklin et al., 2004). The traditional culture-based method underestimated the concentration of the four mould species tested by two to three orders of magnitude. When dust samples were spiked with a known number of mould spores of a particular species, the MSQPCR method quantified the number of spores added within the standard deviation of the assay (Haugland et al., 2004).

MSQPCR is just the first step in creating a "molecular approach" to mould analysis. A combination of assays, using multiple genetic targets, to more precisely subdivide a mould populations based on ecological niches, may be possible. For example, *Penicillium chrysogenum* was found to contain four genetically distinct genotypes (Scott et al., 2004). So MSQPCR is just the beginning of applying a molecular approach to mould analysis. However, practical applications of MSQPCR have already been found by government, commercial, and academic laboratories.

MSQPCR has been used in many applications to assess indoor air and surfaces for moulds. For example, MSQPCR was used by a consulting firm to locate potentially pathogenic *Aspergillus* species during a hospital construction project. Once the mouldy materials were found, they were removed and the area was thoroughly cleaned (Morrison et al., 2004). Follow-up samples, analyzed by MSQPCR, demonstrated to the satisfaction of the hospital management that their new addition was free of these potentially problematic *Aspergillus* species. Other studies measured potentially infectious moulds, e.g., *Candida* and *Aspergillus* species, in water or air samples using MSQPCR (Brinkman et al., 2003; Neely et al., 2004; Vesper et al., 2007c).

MSQPCR has also been used internationally. The National Environmental Agency of Singapore used MSQPCR to evaluate the mould burdens in Singapore shopping centers (Yap et al., 2009d). In a UK survey of moulds in homes, MSQPCR analysis demonstrated that most of the same moulds in the United States were also found in homes across England (Vesper et al., 2005). MSQPCR was used by the National Public Health Institute of Finland to describe the moulds in water-damaged homes in Finland (Lignell et al., 2008). Housing in France

was tested for moulds using MSQPCR (Bellanger et al., 2009) and daycare centers in Sweden (Cai et al., 2009). Even the International Space Station was evaluated using MSQPCR (Vesper et al., 2008b). In addition to public agencies and universities, about a dozen commercial laboratories in the United States, Canada, and Europe, are using MSQPCR.

The environmental relative mouldiness index (ERMI)

A method to compare mould populations in water-damaged and non-water-damaged homes was needed. Initially, it seemed likely that by measuring 82 common mould populations with MSQPCR and summing the total cells would lead to a method to differentiate water damage and home "mouldiness." This was not the case, as you will see below. If total mould populations were not different in water-damaged homes compared to non-water-damaged homes, then it seemed likely that one or more specific moulds would define water-damaged homes, but this was not the case either. Therefore we realized it was combinations of specific mould populations which define a water-damaged home but not always the same combination. In one water-damaged home, it was one set of moulds and in another water-damaged home in was another set of moulds. Ultimately, we were able to compile a set of "indicator" mould species of water damage whose populations could statistically separate a water-damaged home from a non-water-damaged home. Measuring the populations of these "indicator" species resulted in the development of a "Relative Mouldiness Index" (RMI) for homes in Ohio. The application of the RMI approach to a random National sample of homes allowed us to create the "Environmental Relative Mouldiness Index" (ERMI) scale for U.S. homes.

In order to determine what mould populations separated water-damaged and non-water-damaged homes, a study in Cleveland OH, USA was conducted. The homes selected for this Cleveland study were based on an inspection that showed a large area of visible mould. A set of homes in which exhaustive investigation discovered no water or mould problems were used as controls. Dust samples were collected in each of these homes and then analyzed for 82 species of moulds using MSQPCR (Vesper et al., 2004).

It was assumed that the total mould concentrations in these obviously water-damaged homes would be significantly higher than the total in control homes. However, the results indicated that water-damaged homes and control homes could not be statistically distinguished by simply summing the number of mould cells.

Next, I expected that one or more of the moulds would occur in statistically significantly higher concentrations

in water-damaged homes compared to the control homes. No mould species measured by MSQPCR was significantly different in concentration in these obviously water-damaged and mouldy homes compared to the control homes (Vesper et al., 2006). These results lead us to seek an empirical understanding of what defined a water-damaged, mouldy home.

By looking at the columns of data for the population of each of the 82 moulds analyzed, it appeared that there was more "density" to the column of mould population data from water-damaged, mouldy homes compared to the control homes. These density differences were mathematically described by taking the log of the concentration of each mould for each sample.

In addition, about half of the 82 species tested for were rare in occurrence and when they did occur, they were generally found in very low numbers. Only moulds that had a geometric mean of at least 1 cell per mg of dust in these Cleveland homes were selected for grouping. Using the geometric mean as the cut-off, the number of relevant moulds was reduced to 36. Taking the sum of the logs of all 36 species produced a statistically significantly higher value in water-damaged set of homes compared to control homes (Haugland et al., 2005).

It was not any specific mould that distinguished water-damaged and control homes but multiple species. Some species were found in nearly all homes and some appeared to be more common in water-damaged homes. An empirical process was used to organize these groups. If the ratio of the occurrence of a species in water damaged homes to control homes was greater than one, then that mould was associated with "water damage" and put into the "Group 1" moulds. If the ratio was one or less, then that mould species was placed in the "Group 2" moulds, which occur in essentially all homes, independent of water damage.

It was concluded that not only the most abundant moulds were relevant to defining a water-damaged home but also the diversity of moulds present. These 36 species were "indicators" for the occurrence of more than just themselves but for the other rarer moulds as well. Since these 36 species were indicators, the "mould burden" estimate could be adjusted for variations in cleaning habits in different homes based-on differences in accumulation processes for Group 1 versus the Group 2 moulds.

The Group 1 moulds accumulate in the dust based-on indoor mould growing conditions in the home (amount of water, food sources, length of time, etc). But the Group 2 moulds, which primarily come from the outdoors, accumulate in the indoor dust based on the outdoor growing conditions (season, rainfall, vegetation, etc.). In the simplest terms, the accumulations of Group 1 and Group 2 moulds in the dust of homes are governed by different processes. The accumulation of the Group 1 moulds depends on the specific house and its particular water

problem. The Group 2 moulds accumulate in homes from the outside air predominantly. Across the United States, the sum of the logs of the Group 2 species falls between 7 and 14 in about 50% of homes (Vesper 2009a). The result of this realization was a method to adjust homes to a common baseline, eliminating the variability due to cleaning habits.

The significance of the grouping system can be illustrated by the following example. Assume two identical homes with identical water damage and resulting mould problems are tested. In the first house, cleaning had been performed daily and, in the second, cleaning occurred only once a year. If a mould burden estimate was based solely on the 36 species, the mould burden in the two houses would appear to be different. But, for the sake of argument, both homes have identical water and mould problems. By subtracting the sum of the log numbers of the Group 2 moulds from the sum of the logs of the Group 1 moulds, the homes are adjusted to the same "mould baseline."

When this adjustment was made in the study of Cleveland water-damaged versus control homes, the statistical difference in the water-damaged and control homes became even stronger. The result of the subtraction of the sum of the log numbers of the Group 2 species from the sum of the log numbers of the Group 1 species produced a unit-less number called the Relative Mouldiness Index (RMI) value.

The Department of Housing and Urban Development (HUD) sponsored a national survey of homes called the American Healthy Homes Survey (AHHS). A standard dust sample was collected from a random national sampling of 1096 U.S. homes and the RMI value calculated for each home (Vesper et al., 2007b). The RMI values were then assembled on a scale from lowest to highest (Vesper et al., 2007b). In nearly half of the homes, the 82 species (as first tested in Cleveland) were analyzed. None of the additional 46 species met the criteria of a geometric mean concentration of 1 cell per mg dust. Therefore no more species were added to the 36 in the RMI. Once the RMI approach was applied to a randomized sample of
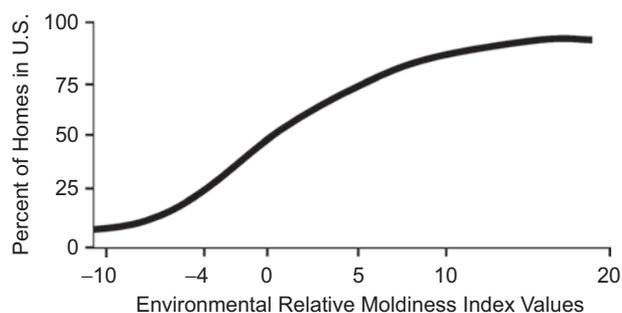
Figure 1. The Environmental Relative Mouldiness Index created by analyzing settled dust in 1096 United States homes.

houses in the US, a scale was created and the index name was changed to the "Environmental Relative Mouldiness Index" (ERMI[SM]) (Figure 1) to describe the National application.

The ERMI was calculated as shown in Equation 1, by taking the sum of the logs of the concentrations of the 26 Group 1 species (s_1) and subtracting the sum of the logs of the concentrations of 10 Group 2 species (s_2) (Vesper et al., 2007b).

$$ERMI = \sum_{i=1}^{26} \log_{10}(S_{1i}) - \sum_{j=1}^{10} \log_{10}(S_{2j}) \qquad (1)$$

The ERMI scale has no units, since it is a relative scale, and is divided into quartiles (Vesper et al., 2007b). The first or lowest quartile indicates the homes with the lowest mould burden. The homes in the fourth or highest quartile (above 5) had the greatest mould burden. The standard deviation of any ERMI value is a maximum of +/– 3 (Vesper et al., 2007b).

Sometimes the standard dust sample cannot be collected, so in 176 of the AHHS homes the vacuum cleaner bag dust was analyzed also. The ERMI values derived from vacuum cleaner dust were about 80% consistent with the standard sample in placing the home into the correct upper or lower 50% of the ERMI scale (Vesper et al., 2009c). In addition to the ERMI analyses of each AHHS home's dust, a traditional inspection and questionnaire was also completed.

In the AHHS study, the inspector in each home made a visual and olfactory investigation for mould. At the same time, the occupants of each home were questioned about water problems or mould in the home in the last 12 months (Vesper et al., 2009c). The ERMI value was found to be in agreement with the inspection and/or occupant's answers about mould and moisture in 48% of fourth quartile homes, but neither of these human assessments indicated a moisture or mould problem in the other 52% of fourth quartile homes (Vesper et al., 2009c). The population of the 26 water-damage indicator moulds was statistically indistinguishable in any of these fourth quartile homes, demonstrating that all of these fourth quartile homes had similar mould-burdens (Vesper et al., 2009c). This demonstrated that the ERMI analysis of a dust sample may reveal "hidden" mould problems in about 50% of these high mould burden homes.

Application of the ERMI to asthma epidemiological studies

Unlike many diseases, asthma does not appear to be caused by a single factor, agent, or exposure. Rather it is a disease that develops over time. On the other hand, exposure to asthma-triggers may be specific to a given child's asthmatic event. Therefore the cause(s) of asthma and the trigger(s) of an asthma event may be the same or different. To determine the cause of asthma, monitoring the air continuously for years may be necessary. Since this is not practical, an alternative source of long-term exposure estimates may be found in the collection and analysis of dust (Chao et al., 2002; Lioy et al., 2002).

Dust samples and the ERMI have been applied in four published asthma studies. In conjunction with HUD, CASE Medical School and the Cuyahoga County Health Department, the moulds in water-damaged homes of asthmatic children were measured by MSQPCR (Haugland et al., 2005). The goal of this study was to define the mould populations in asthmatic children's homes, to remediate these homes for mould by correcting any water problems, removing mouldy materials and thoroughly cleaning the homes and then monitoring the changes in the asthmatic child's health.

Cleveland homes with higher ERMI values were more likely to have an asthmatic child living there (Vesper et al., 2006). The Group 1 moulds were statistically associated with the occurrence of asthma in these children but not the Group 2 moulds (Vesper et al., 2006). Remediation of these homes by fixing the water problem(s) and cleaning the homes resulted in significant reductions in the need for hospital interventions for the children's asthma (Kercsmar et al., 2006). Similar health improvements were reported in a more recent remediation study (Burr et al., 2007).

In a Cincinnati study, infants of atopic parent(s) were the subject of a prospective study of mould and respiratory health. This study showed that the higher the ERMI value in the home at age one, the more likely the infant would develop wheeze and rhinitis between the ages of three to four (Vesper et al., 2007a).

In a Detroit study (Vesper et al., 2008a), the standard dust sample could not be obtained and the dust from the home vacuum cleaner bag was tested. The homes of 83 non-asthmatic children, 28 moderate asthmatic, and 32 severely asthmatic children were tested. Significantly higher ERMI values were measured in the homes of the children with severe asthma compared to those with no asthma (Vesper et al., 2008b).

In a smaller Raleigh study (Vesper et al., 2007c), the ERMI value was calculated from the vacuum cleaner bag dust from the homes of 19 asthmatic children. The ERMI values in these asthmatic children's homes in NC were statistically higher than the ERMI values found in a randomly selected group of homes in the United States.

Conclusions

The medical costs of asthma are approximately $15 billion per year in the United States alone and asthma

results in about 2,000 deaths per year (Fisk et al., 2007). Lost school and work days run into the millions each year. The IOM's expert committee (2004) concluded that exposure to mouldy, damp indoor environments was associated with asthma. A subsequent review (Sahakian et al., 2008) of more recent publications also linked dampness to mould and asthma/asthma symptoms. The World Health organization has come to the same conclusion and suggest that exposure to moulds should be "minimized" (WHO, 2009). A meta-analysis of studies associating mould contamination with adverse health effects demonstrated that building dampness and mould were associated with approximately a 30–50% increase in a variety of respiratory and asthma-related health outcomes (Fisk et al., 2007). Therefore, it is critical that mould assessments are accurate and meaningful.

HUD in its report to Congress (2005) made it clear that the traditional methods of mould sampling, analysis and mould population interpretation were not adequate to answer concerns about mould contamination. The WHO report also described the traditional methods of mould analysis as having "serious flaws" (WHO, 2009). This is supported by the fact that inter-laboratory tests of the same samples using traditional methods produced highly variable and non-interpretable results. On top of that, the typical inspection or occupant questionnaire failed to detect hidden mould problems about 50% of the time (Vesper et al., 2009c).

U.S. EPA researchers developed a DNA-based method of mould analysis called MSQPCR which is sensitive, specific and accurate. The U.S. EPA in conjunction with HUD developed a simple, standard method of sampling homes for mould populations and created a scale called the ERMI to compare the mould burden in homes across the United States (Vesper et al., 2007b).

In four epidemiological studies, higher ERMI values in homes were associated with increased risk of asthma in children. Remediating the water-damage and mould in asthmatics homes resulted in a statistically significant improvement in the child's health and a reduction in the need for hospitalizations and emergency room visits (Kercsmar et al., 2006).

There are limitations in the application of MSQPCR and ERMI. The MSQPCR technology is fairly expensive, especially when you compare it to some of the traditional approaches to mould analysis. In addition, some dust samples have been shown to inhibit the MSQPCR reactions but these are generally rare samples (Vesper et al., 2007b). Heavy concentrations of gypsum and cement dust should be specifically avoided. Another limitation of MSQPCR is that the only moulds measured are those selected by choice of assays and, of course, not every mould has a MSQPCR assay at this time. As always, even though many of the human elements have been removed from the technology and there are

internal controls, human errors are still a possibility with MSQPCR.

The ERMI is susceptible to a number of confounders. Although the dust sampling procedure is fairly simple, an untrained person might do it improperly. Significant changes in the home conditions (new carpet, recent pipe leak, and so on) immediately before sampling could result in a sample that does not represent the previous or long-term mouldiness of the home. The ERMI is also not a method to separate homes with fairly small differences in mouldiness because the ERMI value is affected by the analytical variability in up to 36 different analyses that make up the ERMI value. Although the ERMI is based on a single standard sample from a home, other settled-dust samples (from the basement, attic, and so on) can be analyzed the same way. These results may help to locate the source of the problem.

Even with all of the limitations of MSQPCR and the ERMI, these technologies may provide a more scientifically sound approach to mould analysis and data interpretation than has been available using traditional methods. However, these developments are only the first steps in building methodologies which will eventually provide a comprehensive picture of the mould populations in homes and eventually in other buildings.

Notice

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Declaration of interest

The United States Environmental Protection Agency (US EPA) owns the patent for MSQPCR and therefore any commercial application can result in royalties to the US EPA and the inventors (Richard Haugland and Stephen Vesper).

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