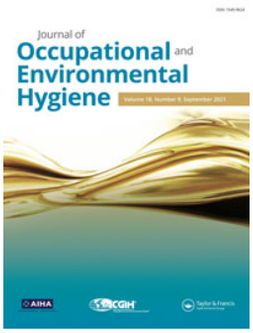

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SHORT REPORT

Comparison of ERMI results for dust collected from homes by an electrostatic cloth and by the standard vacuum method

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ABSTRACT

The Environmental Relative Moldiness Index (ERMI) is a scale used to compare mold contamination levels in U.S. homes. To create the ERMI scale, a nationally representative set of U.S. homes was selected (n = 1,096). From each of these homes, a standard vacuum-dust sample was collected and then 36 common molds, the 26 Group 1 and 10 Group 2 molds, as grouped for forming the ERMI metric, were quantified using quantitative PCR assays. However, in investigations of mold in homes, it is not always practical or even possible to collect dust using the standard vacuum method. Therefore, we performed a comparative study of dust samples collected in the same homes (n = 151) by the standard vacuum method and by an electrostatic cloth (EC) method. First, floor dust was collected by vacuuming a 2 m^2 area in the living room and a 2 m^2 area in a bedroom, directly adjacent to the sofa or bed, for 5 min each with a Mitest sampler-fitted vacuum. Second, immediately after the collection of the vacuum dust sample, an EC dust sample was collected by wiping above-floor horizontal surfaces in the living room and bedroom. Then, the ERMI analysis of each sample was performed by a commercial laboratory. The results showed the average concentrations of 33 of the 36 ERMI molds were not significantly different in the vacuum and EC samples. Also, the average summed logs of the Group 1 molds, Group 2, or ERMI values were significantly ($p < 0.001$) correlated between the vacuum and EC samples. Logistic regression indicated that an EC sample could identify homes in the highest ERMI quartile 96% of the time by using the same ERMI value cutoff as vacuum sample ERMI value cutoff and 35% of samples proved to be false positives. When it is not practical to obtain the standard vacuum-dust sample, an EC sample can provide a useful practical alternative for ERMI analyses.

KEYWORDS

American Healthy Homes Survey; Department of Housing and Urban Development; mold

Introduction

Reviews of the scientific literature have concluded that exposure to high levels of mold contamination is associated with adverse health effects, especially for people with asthma (WHO 2009; Kanchongkittiphon et al. 2015; Osborne et al. 2015; Thacher et al. 2017; Mendell et al. 2018). Therefore, standardized and objective methods are required to quantify mold contamination in homes. To fill this need, the Environmental Relative Moldiness Index (ERMI) was created (Vesper et al. 2007).

The ERMI scale was produced using data from the Department of Housing and Urban Development's (HUD) first American Healthy Homes Survey (AHHS I) (Vesper et al. 2007). Standard vacuum dust samples were collected from a nationally representative selection of U.S. homes (n = 1,096) and each sample was analyzed using qPCR assays for the 36 molds that make up the ERMI metric. The final ERMI scale ranged from about a −10 to about 20 (Vesper et al. 2007). Homes with an ERMI value >5 are in the highest quartile and most likely to be associated with asthma (Vesper and Wymer 2016).

The dust collection method used by HUD in AHHS I required vacuuming a 2 m^2 area in the living room and a 2 m^2 area in a bedroom, directly adjacent to the sofa and bed, for 5 min each with a Mitest sampler-fitted vacuum (Vesper et al. 2007). We recognize there are times when collecting such a sample is not practical or even possible. For example, if there is no

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furniture in the home, one cannot locate the previous position of the sofa and bed. (The standard method requires vacuuming the 2 m^2 area adjacent to the sofa and bed.). Other reasons for using a practical alternative method of dust collection, include a sampler's need to collect samples quickly and study participant's/homeowner's sample self-collection.

Therefore, we looked for a practical alternative method of dust collection for the ERMI analysis, although not standardized could still be useful.

Cox et al. (2017) demonstrated that the mold collected with an electrostatic cloth (EC), either actively wiping above floor surfaces or passively collecting dust by leaving an EC for an extended period in the home, provided mold-level estimates comparable to that provided by vacuum-collected floor dust. However, it is not always possible to leave an EC cloth for an extended time. Any practical alternative dust collection method must be simple and rapid. Our objective was to compare results of the ERMI analyses of EC and vacuum-collected dust and to determine the consistency between EC and vacuum samples in placing homes in the highest ERMI quartile, i.e., homes with ERMI values >5.

Methods

Home selection process

In 2019, HUD completed the second American Healthy Homes Survey (AHHS II). The same selection process was used to select the homes for AHHS II as was used in AHHS 1 (see Vesper et al. 2007 for the detailed procedure.). Briefly, the selection process was based on the 2010 U.S. Census for homes in the continental U.S. The first step identified 79 clusters called "primary sampling units" (PSUs), with a probability proportional to population in the U.S. Census. The second step chooses five "segments" in each PSU with probability proportional to the total number of housing units in the segment, which is a Census block or a group of blocks. The third step was the identification of "sampling frames," which are a list of all housing units in the segments from which the sample of housing units were drawn. A frame of housing units was created by a variation of a process called "listing." Listing is the process of identifying and recording the addresses of households on listing sheets. In this way, all places where people live, or might live, within the boundaries of the segment are eligible for inclusion in the study. From these listings, the homes for AHHS II (n = 695) were selected (Vesper et al. 2021).

During 2018 and into early 2019, the sampling teams traveled to the identified regions across the U.S. to reach the selected homes (n = 695). The sampling in each home was completed at any time during the study based on when the homes became accessible. The selection of homes for EC sampling was based on the instruction to the sampling teams to collect the EC samples in the first two homes in each "primary sampling unit" PSU. In seven cases, the sample-taker forgot to obtain an EC sample and so only 151 EC samples were obtained instead of 158.

Dust sample collection

Dust samples were collected for this study from AHHS II homes (n = 151) in two ways. First, dust from each home was collected by vacuuming a 2 m^2 area in the living room and a 2 m^2 area in a bedroom, directly adjacent to the sofa or bed, for 5 min each with a Mitest sampler-fitted vacuum, exactly as performed in AHHS I. Second, immediately after the collection of the vacuum dust sample, an EC dust sample was collected. A gloved hand was used to wipe above-floor horizontal surfaces in the living room and bedroom, e.g., door frames, bookshelves, and windowsills, with an EC (Swiffer, Proctor and Gamble, Cincinnati, OH) until the white cloth became gray with dust. The cloth was then placed in a sealable plastic bag (Ziplock, SC Johnson, Racine, WI). The samples were placed in a cooler (4 °C) and returned to the laboratory where they were stored at −20 °C until analyzed.

Quantitative PCR (qPCR) analysis

The vacuum dust was recovered from the MiTest collector, as previously described (Vesper et al. 2007) and the EC dust was recovered, as previously described (Cox et al. 2017). Five milligrams of sieved (pore 300 µm) dust from each sample was added to a 2-mL extraction tube containing 0.3 g of glass beads, as previously described (Haugland et al. 2004). Each EC or vacuum dust sample was spiked with 1×10^6 conidia of *Geotrichum candidum* at the time of extraction as an internal reference to ensure that the extraction and purification were performed correctly (Haugland et al. 2004). A bead beater (Biospec Products, Bartlesville, OK) was used to shake each extraction tube at 5,000 rpm for 1 min to release the DNA from the cells. The DNA was then purified using the DNA-EZ extraction kit (GeneRite, Monmouth Junction, NJ), following the manufacturer's instructions.

Table 1. Average (AVG) concentration and standard deviation (SD) in cell equivalents per mg dust (CE/mg dust) of each of the 36 Environmental Relative Moldiness Index (ERMI) molds in dust samples collected by electrostatic cloth (EC) or vacuum from the same homes (n = 151) compared using the Wilcoxon rank sum test, corrected for multiple comparisons using the Holms–Bonferroni test.

Group 1 Molds	Swiffer AVG CE/mg dust	SD	Floor AVG CE/mg dust	SD	Wilcoxon *p*-value
Aspergillus flavus	9.6	38	2.1	6	0.017
Aspergillus fumigatus	12.3	36	5.5	18	0.041
Aspergillus niger	261.1	733	98.9	360	0.015
Aspergillus ochraceus	21.3	69	43.4	335	0.428
Aspergillus penicillioides	11,353.8	65,219	1534.5	6505	0.067
Aspergillus restrictus	183.0	1167	65.0	332	0.233
Aspergillus sclerotiorum	4.1	13	5.0	22	0.643
Aspergillus sydowii	157.8	374	28.0	66	**<0.001**
Aspergillus unguis	6.0	16	3.5	26	0.316
Aspergillus versicolor	229.2	496	391.1	2583	0.450
Aureobasidium pullulans	756.6	2465	710.7	1187	0.837
Chaetomium globosum	48.0	138	7.4	21	**<0.001**
Cladosporium sphaerospermum	1858.1	16,307	264.6	883	0.231
Eurotium amstelodami	1999.3	10,901	573.8	1914	0.115
Paecilomyces variotii	15.8	57	11.3	44	0.445
Penicillium brevicompactum	28.6	55	28.7	112	0.992
Penicillium corylophilum	80.6	282	48.5	328	0.362
Penicillium crustosum	77.6	229	56.7	296	0.492
Penicillium purpurogenum	2.0	4	2.2	20	0.887
Penicillium spinulosum	0.3	1	0.3	3	0.976
Penicillium variabile	69.5	243	13.2	26	0.005
Scopulariopsis brevicaulis	19.9	92	9.2	43	0.192
Scopulariopsis chartarum	6.9	17	9.6	38	0.440
Stachybotrys chartarum	10.2	29	1.5	4	**<0.001**
Trichoderma viride	21.2	129	11.6	42	0.389
Wallemia sebi	4803.0	39,557	5236.5	33268	0.918
Group 2 Molds					
Acremonium strictum	17.3	9	46.0	159	0.033
Alternaria alternata	354.4	43	181.2	372	0.006
Aspergillus ustus	34.7	667	6.1	22	0.125
Cladosporium cladosporioides Type 1	2271.0	228	1588.7	2127	0.408
Cladosporium cladosporioides Type 2	263.8	9897	52.2	127	0.120
Cladosporium herbarum	1703.5	1663	814.6	1516	0.010
Epicoccum nigrum	276.4	3906	234.4	444	0.401
Mucor amphibiorum	94.9	426	220.8	935	0.118
Penicillium chrysogenum Type 2	377.1	311	610.5	4251	0.526
Rhizopus stolonifer	37.3	1526	14.6	59	0.031

Significant differences are bolded.

Each of the 36 ERMI molds was quantified in each extract with qPCR assays described earlier (Haugland and Vesper 2002). The standard qPCR assay contained 1 μL of a mixture of forward and reverse primers at 25 μM each, 12.5 μL of "Universal Master Mix" (Applied Biosystems Inc., Foster City, CA), 2.5 μL of 2 mg mL^{-1} fraction V bovine serum albumin (Sigma Chemical, St. Louis, MO), 2.5 μL of a 400 nM TaqMan probe (Applied Biosystems Inc., Foster City, CA), and 2.5 μL of DNA free water (Cepheid, Sunnyvale, CA). Five microliter of the DNA extract from the sample and this mix were combined. Reactions were performed with thermal cycling conditions consisting of 2 min at 50 °C, 10 min at 95 °C, followed by 40 cycles of 15 sec at 95 °C for template denaturation and 1 min at 60 °C for probe and primer annealing and primer extension, and 5 mg from each of the sieved-dust samples were analyzed by a commercial laboratory that performs the ERMI analysis (Mycometrics LLC, Monmouth Junction, NJ).

Environmental Relative Moldiness Index (ERMI) calculation

The ERMI metric is based on the analysis of 36 widely distributed indicator molds: 26 Group 1 molds, which were associated with water-damage in homes, and 10 Group 2 molds, which primarily enter the home from the outside environment (Vesper 2011). After the concentrations (cell equivalents per mg of dust) of each of the 36 ERMI molds were determined, the ERMI values were calculated, as shown in Equation 1. The summed common logs of the concentrations of the Group 2 molds (s_2) was subtracted from the summed common logs of the concentrations of Group 1 molds (s_1) to produce the ERMI value (Vesper et al. 2007):

Table 2. Pearson correlations of ERMI metrics for electrostatic cloth (EC) and standard vacuum dust samples for their average (AVG) summed logs of Group 1 molds, average summed logs of Group 2 molds, or average Environmental Relative Moldiness Index (ERMI) values (SD = standard deviation).

	EC Dust		Vacuum Dust		Pearson	
	AVG	SD	AVG	SD	Correlation	*p*-value
Sum logs Group 1	27.1	8.1	20.6	9.0	0.57	<0.001
Sum logs Group 2	15.9	4.6	13.9	4.3	0.61	<0.001
ERMI	11.2	6.4	6.7	6.9	0.64	<0.001

Significant correlations are bolded.

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

This approach to mold quantification was used because of the great variability in mold populations. Therefore, logs were used. In addition, a normalization for differences in the outdoor environments, cleaning habits and types of ventilation, led us to subtract Group 2 from Group 1 mold populations (for a more detailed explanation, see Vesper 2011).

Statistical analyses

The average concentration of each of the 36-ERMI molds in the vacuum dust and the EC dust samples were compared using the Wilcoxon rank sum test, corrected for multiple comparisons using the Holms–Bonferroni test. The Pearson correlation test was used to determine the correlation between the summed logs of the Group 1 and Group 2 molds and the ERMI values in the vacuum dust and the EC dust samples.

For the EC sample ERMI values, the rates of "true positive" and "true negative" concurrence with the vacuum dust sample results were calculated using logistic regression of the EC sample results as predictors for the corresponding ERMI values from the vacuum dust samples. Homes with vacuum dust ERMI values >5 were defined as "true" 4th quartile homes on the ERMI scale. Receiver operating characteristic (ROC) analysis was used, in conjunction with the Youden's index, to identify all points on the ROC curve (Youden 1950; Schisterman et al. 2005).

The Youden Index is a way of summarizing the performance of a "diagnostic" test, which, in this case, is the test of the EC results compared to the vacuum dust sample results. The Youden Index value ranges from 0 through 1. A Youden Index value of 0 means the test is useless. The closer to a value of 1, the more useful is the test. As is a common practice, the maximum value of the Youden index was used to identify the optimum ERMI cutoff value, which maximizes the sum of the proportions of "true positives" and "true negatives" (Youden 1950). Statistical analyses and graphics were performed using SAS version 9.4 (SAS Institute Inc., Cary, NC).

Results

Table 1 shows the comparison of the average concentration of each of the 36 ERMI molds in the vacuum dust and EC dust samples. The average concentration of 33 of the 36 ERMI molds were not significantly different in the vacuum and EC dust samples. Only the average concentrations of three molds (*Aspergillus sydowii*, *Chaetomium globosum*, and *Stachybotrys chartarum*) were significantly ($p < 0.001$) different in EC dust compared to vacuum dust samples.

For the vacuum and EC dust samples, Table 2 shows the Pearson correlation between components of the ERMI metric (summed logs Group 1 molds and summed logs of the Group 2 molds) and the ERMI values themselves. The Group 1, Group 2, and ERMI values were each significantly ($p < 0.001$) correlated between the vacuum dust and EC dust samples.

Based on the ROC curve analysis, the optimum ERMI cutoff value was determined to be an ERMI value of 9.6 (Figure 1). The associated Youden index had a predictive rate of 80% for "true positives" and 72% for "true negatives." By lowering the cutoff to an ERMI of 5.0 for EC samples, i.e., the same cutoff value used for standard vacuum samples, "true positives" were identified 96% of the time. Therefore, the probability of misidentifying the highest ERMI quartile homes was reduced from 20% to only 4%.

Discussion

The average concentration of 92% of the 36-ERMI molds were not significantly different in the vacuum and EC dust samples from the same homes. Also, the sum logs of the Group 1, Group 2, and ERMI values in the vacuum and EC samples were significantly correlated. Therefore, the ERMI analysis was functioning in a consistent manner for both vacuum and EC samples and, thus, the EC and vacuum sample ERMI results could be compared using ROC curves to estimate the accuracy of the EC sample, much like as done for a medical diagnostic test, by calculating a Youden index value (Mallett et al. 2012).

The ROC curve was constructed by plotting the true positive rate against the false positive rate of agreement between observations. The true positive is the proportion of observations that were correctly

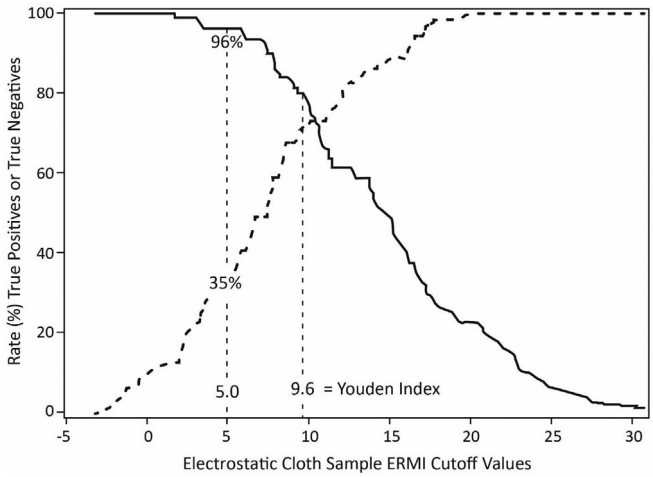

Figure 1. Results of the logistic regression analysis to determine the rates of "true positive" and "true negative" for accurately categorizing high quartile Environmental Relative Moldiness Index (ERMI) homes, based on electrostatic cloth sample ERMI values >5. The solid line shows the true positive rate and the dashed line the true negative rate of agreement.

predicted to be positive out of all positive observations and the false positive rate is the proportion that incorrectly predicted to be positive out of all the negative observations. The closer the curve is to the left-hand border and the top border, the more accurate is the test. Therefore, the greater the area under the curve, the more useful is the test.

The area of the ROC curve in this study was found to be 0.75 and this value would be considered "good" in an evaluation of a medical diagnostic test (Šimundić 2009; Wolk 2020). An ERMI cutoff value for EC samples can be set at the same value used for vacuum samples, indicating a high rate of identifying homes with a higher likelihood for major mold contamination, i.e., ERMI values >5. Finding these homes with high ERMI values is most important because epidemiological studies of asthma and home ERMI values demonstrated that the higher the ERMI value, the greater was the likelihood of occupant asthma (Vesper and Wymer 2016).

ECs have been used in previous studies to collect mold, allergens, endotoxin, etc. (Shorter et al. 2018; Kristono et al. 2019; Viegas et al. 2020). However, in these studies, the EC cloth collected newly settled dust by leaving the cloth for an extended period to passively collect the dust. However, it is important to test the EC collection method for the specific application. Some allergens were found to be differentially collected by EC and vacuum sampling (Kristono et al. 2019). For example, the concentrations of Fel d I were strongly correlated in samples collected by EC and vacuum but not endotoxin or Der p I (Kristono et al. 2019).

Direct comparison of dust samples collected using EC and vacuum dust have shown good correlations for ERMI values (Cox et al. 2017). In our study, there was a tendency for the EC sample to produce higher ERMI values than the comparable vacuum samples. This may be a result of the differences in the charge (positive or negative) of particles collected by vacuuming, which would collect all particles, compared to wiping with the EC cloth, which might show a charge preference. Despite this tendency, the EC method of dust sampling appears to be a useful practical alternative for the standard vacuum sample, when that type of sample is not available.

Obtaining the HUD standard vacuum dust sample is not always practical or possible. In some cases, the home to be investigated for mold contamination is devoid of furniture. In other cases, it may not be practical for the occupant to have someone come into the home to obtain the vacuum sample. Since the collection method is simple and the EC cloths are readily available, a protocol could be developed for having the EC sample obtained by the occupant. However, we do recognize that there are limitations in this study to consider.

The major limitation of this study was the small number of homes tested, i.e., only 22% of the total number of homes in AHHS II and only 14% of the total number of homes in AHHS I. Also, unlike the very prescriptive protocol for obtaining the standard vacuum dust sample, the EC sample collection protocol is very home and situation specific. Therefore, the EC method described here is not a standardized method of dust collection, but only a practical

alternative, which may be useful when the standard method cannot be used. The EC method described here is not a replacement for the standard vacuum sampling method. It might be possible to create a standardized EC sampling method by using a defined area of floor to wipe with an EC cloth, but this was not our goal. Also, additional studies comparing "actively" collected EC samples and "passively" collected EC samples would be useful to consider in the future. Despite these limitations, the EC sample can be a useful practical alternative for the standard vacuum dust sample when trying to identify homes in the highest ERMI quartile.

Conclusions

When it is not practical or possible to obtain the standard vacuum dust sample for the application of the ERMI analysis, the collection of dust using an EC appears to be a practical alternative for identifying homes in the highest ERMI quartile, since a dust sample collected by the standard vacuum method or by EC agreed 96% of the time for placement of a home in the highest quartile on the ERMI scale.

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Conflicts of interest

There are no additional conflicts to declare.

Disclaimers

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