Screening Tools to Estimate Mold Burdens in Homes

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Objective: *The objective of this study was to develop screening tools that could be used to estimate the mold burden in a home which would indicate whether more detailed testing might be useful.* ***Methods:*** *Two possible screening methods were considered for mold analysis: use of vacuum cleaner bag dust rather than the standard protocol dust samples and reducing the number of molds needed to be quantified resulting in the creation of an alternative mold burden scale.* ***Results:*** *Vacuum bag dust analysis placed the estimate of mold burden into the upper or lower half of the Environmental Relative Moldiness Index scale. Mold burdens estimated by only 12 species produced an index, the American Relative Moldiness Index, with a correlation of* $\rho = 0.80$ with the Environmental Relative Moldiness Index. ***Conclusions:*** *Two screening tools were developed for estimating the mold burden in homes.* (J Occup Environ Med. *2009;51:80–86*)

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Sampling for molds in the environment has traditionally been done with short air samples that were analyzed by either microscopic observation and counting or culturing followed by microscopic speciation. In either case, the results were dependent on the skill and knowledge of the analyst. Furthermore, the interpretation of the results was then left up to the individual because there was no objective method of standardization. An Institute of Medicine report suggested that what was needed was a molecular method of mold analysis.[1]

In 2002, the US Environmental Protection Agency patented such a molecular method called mold specific quantitative PCR (MSQPCR).[2] This technology uses a DNA-based method of identification and an instrument called a sequence detector for quantification. Assays were developed for over 130 of the most common indoor mold species ("EPA Technology for Mold Identification and Enumeration" available at: http://www.epa.gov/microbes/moldtech.htm). The results generated by the MSQPCR process have created the possibility of objectively describing the mold burden in a home.

The 2006 American Healthy Home Survey (AHHS) was completed by the Department of Housing and Urban Development. During the AHHS, dust samples (1096) were collected according to a sampling protocol referred to as the standard dust sample (SD) which entails vacuuming 2 m^2 in the living room and 2 m^2 in a bedroom.[3] The samples were analyzed for the 36 indicator

species, ie, the 26 group 1 species associated with water damage and 10 group 2 species commonly found in homes, with or without water-damage.[3] These results were used in the development of the Environmental Relative Moldiness Index (ERMI) scale.[3] The ERMI scale allows homes to be ranked in terms of relative water-damage and mold growth and allows homes to be divided into groups on the basis of rank order statistics such as at the median or at the upper and lower quartiles.[3]

In this current study, we sought to develop screening tools that might make samples easier to obtain or less expensive to process, yet still be useful for initial identification of homes with potentially problematic water and mold problems. These screening tools were tested on the criterion of how well they put homes into the same rank order categories as the ERMI.

Materials and Methods

Our initial studies of indoor molds focused on 82 species frequently mentioned in the scientific literature. Of the 82 species analyzed in samples of water-damaged and control homes, only 36 species were widely distributed. These 36 species were divided into 26 group 1 species associated with water damage and 10 group 2 species that are not associated with water damage.[3] The other 46 species (listed in Table 1) from the original 82 did not appear to be common but to test this nationally, 455 of the 1096 dust samples from the AHHS were quantified using MSQPCR.[4–6] All primer and probe sequences, as well as known species comprising any assay cluster were published at the web site: "EPA Technology for Mold Identification and Enumeration" available at: http://www.epa.gov/microbes/moldtech.htm.

In addition to the SD sample collected from each home, the AHHS collection team obtained a vacuum cleaner bag from 157 of the survey homes (referred to as VB samples).

TABLE 1
Additional 46 Mold Species/Clusters Measured in AHHS Dust Samples

Mold Species/Clusters	Occurrence (%)	Mean (CE mg^{-1})	GM (CE mg^{-1})	Highest (CE mg^{-1})
Aspergillus caespitosus	1	0	0	2
Aspergillus candidus	36	3	1	396
Aspergillus carbonarius	1	0	0	3
Aspergillus cervinus	0	0	0	0
*Aspergillus clavatus**	1	0	0	24
Aspergillus flavipes	1	0	0	7
Aspergillus niveus	0	0	0	0
Aspergillus paradoxus	1	0	0	7
Aspergillus parasiticus	1	0	0	11
Aspergillus puniceus	5	0	0	29
Aspergillus tamari	6	1	0	40
Aspergillus terreus	32	3	2	313
Aspergillus wentii	1	0	0	106
Emericella nidulans†	10	1	0	134
Emericella variecolor	0	0	0	0
Memnoniella echinata	1	0	0	10
Penicillium atramentosum	14	12	2	3143
Penicillium group 1‡	13	4	1	746
Penicillium canescens	11	3	1	123
Penicillium citreonigrum	27	9	2	177
Penicillium citrinum§	4	3	1	486
Penicillium coprophilium	0	0	0	0
Penicillium decumbens	0	0	0	0
Penicillium digitatum	12	2	0	288
Penicillium expansum	3	1	0	88
Penicillium fellutanum‖	33	14	2	2039
Penicillium glandicola	1	0	0	23
Penicillium griseofulvum	11	2	0	586
Penicillium implicatum	5	0	0	34
Penicillium islandicum	3	1	0	246
Penicillium italicum	0	0	0	0
Penicillium melinii	0	0	0	0
Penicillium miczynskii	0	0	0	0
Penicillium olsonii	20	5	1	387
Penicillium oxalicum	32	13	2	908
Penicillium raistrickii	3	0	0	20
Penicillium restrictum	2	0	0	13
Penicillium roquefortii	6	2	0	426
Penicillium sclerotiorum	15	1	0	15
Penicillium simplicissimum¶	7	3	0	625
Trichoderma asperellum#	12	1	1	233
Trichoderma harzianum	6	2	1	412
*Trichoderma longibrachiatum***	17	2	1	309
Ulocladium atrum	9	1	0	249
Ulocladium botrytis	9	2	0	522
Ulocladium chartarum	2	0	0	37

*Includes *A. clavatus* and *A. giganteus.*
†Includes *E. nidulans*, *E. quadrilineata,* and *E. rugulosa.*
‡Includes *P. aurantiogriseum*, *P. freii*, *P. hirsutum*, *P. polonicum, P. tricolor*, *P. verrucosum,* and *P. viridicatum.*
§Includes *P. citrinum*, *P. sartoryi,* and *P. westlingi.*
‖Includes *P. fellutanum* and *P. charlesii.*
¶Includes *P. simplicissimum* and *P. ochrocloron.*
#Includes *T. asperellum* and *T. hamatum.*
**Includes *T. longibrachiatum* and *T. citrinoviride.*
Percent occurrence in the homes, Mean and GM in CE mg^{-1}, and highest concentration CE mg^{-1} measured in 455 of the 1096 homes.

Dust from bagged or bag-less vacuum cleaners was placed in polypropylene zip top collection bags. VB samples were gamma irradiated until each received a total minimum dose of 2.5 millirads. Individual dust samples were transferred from the VB (or polypropylene zip top bags, in the case of samples from bag-less vacuum cleaners) into a clean sieve-stack equipped with a clean stainless steel bottom collection unit and lid. This assembly was placed in a Syntron Jogger J-1 Sieve Shaker (Syntron, Bioresearch, Carlsbad, CA) and the unit was operated to at least 75% sieve energy for 30 to 45 minutes. Then 5.0 ± 0.1 mg of sieved dust was extracted as described previously.[3] Mold concentration values that were below the minimum detection limit of 1 cell equivalent unit per mg dust (CE mg^{-1}) were treated as left-censored data and all summary statistics referenced were estimated using a modified Kaplan-Meier survival model adapted for left-censored data.[7]

In order to develop a reduced mold panel, an iterative stepwise process was used to reduce the 36 species mold panel used to compute the ERMI to a smaller subset of species, while maintaining a high correlation with the ERMI. Data from SD samples from the 1096 homes surveyed in the AHHS were used to develop a new index, the American Relative Moldiness Index (ARMI). The process followed the general format of a stepwise regression, ie, the process starts with all possible species. A selected mold species is added to another and then another while monitoring, by regression, the correlation between the ERMI and each possible new ARMI value. A mold species was added or dropped from the ARMI calculation depending on whether it improved the correlation of the ARMI to the ERMI values with the fewest number of species.

To test these two screening tools, VB and SD samples were obtained from 21 additional homes. The summary statistic used for testing was the median rank classification which is coded 1 for a home sample below the sample median of all 21 homes, and 2 if above the median. The two proposed screening tools were judged on their consistency in classifying test homes at the same median rank as the ERMI computed from the SD sample taken as the reference. The ERMI values from the VB samples were compared to the ERMI values from SD samples, and the ARMI and ERMI values were compared from the same SD sample. All statistical analyses and graphics were performed using the SAS software system (version 9.1, SAS Institute Inc., Cary, NC) and the R Software environment for statistical computing and graphics (version 2.5, http://www.r-project.org).

Results

The occurrence rates of the additional 46 species analyzed in about half of the AHHS SD samples indicated that these mold species were fairly rare (Table 1). Even the highest

TABLE 2
HUD Survey Geometric Means and Standard Deviations for 1096 Homes Using the Standard Dust Samples

Mold Species	Geometric Mean (CE mg^{-1})	Standard Deviation (CE mg^{-1})
Group 1		
Aspergillus flavus	2.01	3.86
Aspergillus fumigatus	2.99	3.89
Aspergillus niger	3.55	4.65
Aspergillus ochraceus	2.10	4.20
Aspergillus penicillioides	90.43	16.18
Aureobasidium pullulans	262.96	9.24
Aspergillus restrictus	1.66	4.39
Aspergillus sclerotiorum	1.56	2.70
Aspergillus sydowii	2.91	6.36
Aspergillus unguis	1.54	3.11
Aspergillus versicolor	2.41	5.06
Chaetomium globosum	2.33	3.85
Cladosporium sphaerospermum	13.47	7.78
Eurotium group	153.22	8.69
Penicillium brevicompactum	5.48	8.58
Penicillium corylophilum	1.69	3.81
Penicillium group 2	1.27	2.71
Penicillium purpurogenum	1.17	1.70
Penicillium spinulosum	1.40	2.39
Penicillium variabile	3.44	4.85
Paecilomyces variotii	2.40	4.29
Scopulariopsis brevicaulis	2.42	3.84
Scopulariopsis chartarum	1.57	2.49
Stachybotrys chartarum	2.26	4.65
Trichoderma viride	1.51	2.35
Wallemia sebi	17.64	10.74
Group 2		
Alternaria alternata	34.68	7.42
Acremonium strictum	4.09	4.62
Aspergillus ustus	1.77	2.91
Cladosporium cladosporioides type 1	333.71	6.21
Cladosporium cladosporioides type 2	4.18	4.96
Cladosporium herbarum	30.74	12.35
Epicoccum nigrum	118.10	14.47
Mucor group	15.58	6.64
Penicillium chrysogenum Type 2	5.50	6.55
Rhizopus stolonifer	1.35	2.17

Mold species used for the American Relative Moldiness Index are shown in bold type.

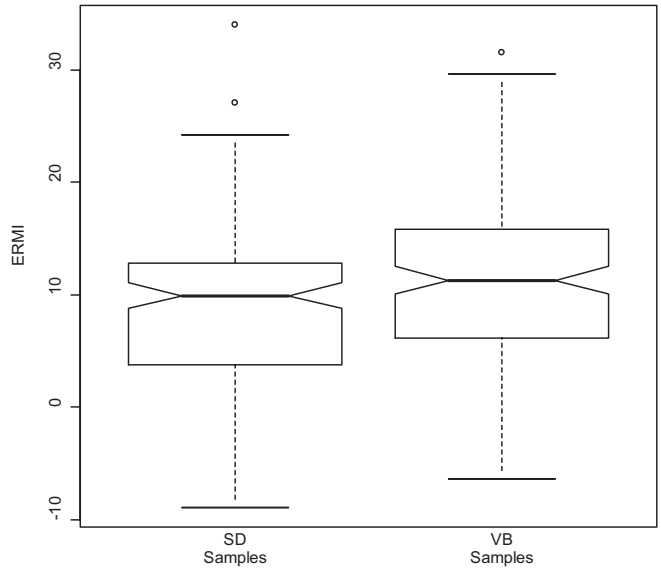

Fig. 1. Box-plots comparing vacuum bag (VB) Environmental Relative Moldiness Index (ERMI) values to standard dust (SD) ERMI values.

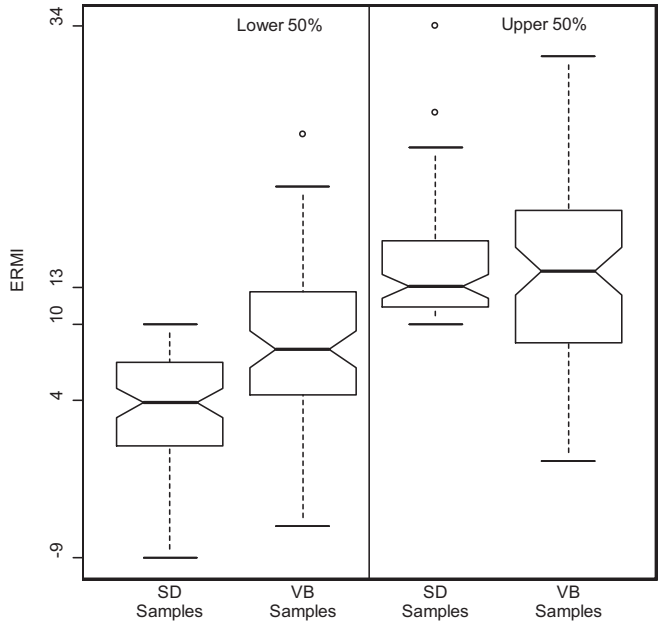

Fig. 2. Box-plots of VB dust versus the SD sample in the upper and lower halves of the Environmental Relative Moldiness Index (ERMI) scale.

spore concentration measured in any sample indicated relatively low concentrations as compared with the 36 indicator species.[3] In addition, almost all of these 46 species occurred in concentrations below the minimum detection limit in more than 50% of sampled US homes (Table 1) as compared with the 36 indicator species currently used to calculate the ERMI (Table 2). Thus, it was determined that the original 36 ERMI species were sufficient to characterize the mold burden of homes and to test the two screening approaches.

Box-plot comparisons of ERMI values calculated from the paired SD and VB samples in 157 homes indicated that overall the average ERMI values were greater in the VB compared with the SD samples (Fig. 1). However, the VB and SD were fairly consistent in categorizing a home in the upper 50% or lower 50% on the ERMI scale (Fig. 2).

Comparison of the geometric means (GM) of individual mold species in the VB versus the SD samples (Table 3) showed that the VB samples had a higher GM than the SD sample for 14 of 26 group 1 species, and 3 of 10 group 2 species. Only 8/26 group 1 species and 2/10 group 2 species had GM that were not statistically different in the two types of samples.

In the set of 21 newly tested homes (Table 4 and Fig. 3), the ERMI values calculated from the VB and SD paired samples were in agreement in placing a home above or below the median in 15 of 21 cases (71%). The odds ratio for consistent placement was 5.6 and on the margin of significance by Fisher exact test ($P = 0.086$). This demonstrates that the ERMI value calculated from the VB sample from a home would be consistently classified with the SD sample at the same median rank at a ratio of about 5.6:1.

The stepwise process used to reduce the number of species assayed resulted in the ARMI scale that could be computed on only 12 species, ie, 9 from group 1 and 3 from group 2 (Table 3) and maintained a correlation with the ERMI of 80%. In the set of 21 newly tested homes (Table 5 and Fig. 4), the results show that the ARMI and the ERMI fell on the same side of the median in 17 of 21 test homes. The odds ratio for consistent placement for the ARMI value was 14.9 which is significant by Fisher exact test ($P = 0.009$) suggesting the ARMI value would be consistent with the ERMI value in placing a home at the same median rank (ie, above or below the median) at a ratio of about 14.9:1.

Discussion

In order to create the ERMI scale, a standardized method of dust sampling, preparation and analysis was developed for use with a nationally representative sampling of US homes. The ERMI scale was based

TABLE 3
Comparison of American Healthy Home Survey Results for Geometric Means and Standard Deviations for 157 VB Dust and SD Samples

Mold Species	VB Detect Rate (%)	SD Detect Rate (%)	Geometric Mean VB/SD	Rank-sum test *P*
Group 1				
Aspergillus flavus	49	46	0.70	0.001
Aspergillus fumigatus	49	73	0.41	<0.001
Aspergillus niger	86	82	1.19	0.053
Aspergillus ochraceus	73	48	8.47	<0.001
Aspergillus penicillioides	92	99	0.53	0.05
Aureobasidium pullulans	81	96	0.92	0.457
Aspergillus restrictus	56	24	1.18	0.108
Aspergillus sclerotiorum	59	44	1.34	0.014
Aspergillus sydowii	31	41	0.35	<0.001
Aspergillus unguis	82	29	2.46	<0.001
Aspergillus versicolor	52	47	1.05	0.698
Chaetomium globosum	64	55	0.85	0.43
Cladosporium sphaerospermum	96	89	1.18	0.22
Eurotium group	93	97	0.37	<0.001
Penicillium brevicompactum	87	66	1.49	0.026
Penicillium corylophilum	41	28	0.82	0.128
Penicillium group 2	41	12	2.19	<0.001
Penicillium purpurogenum	50	18	1.61	<0.001
Penicillium spinulosum	69	30	3.06	<0.001
Penicillium variabile	80	61	0.59	<0.001
Paecilomyces variotii	78	61	1.13	0.325
Scopulariopsis brevicaulis	48	61	0.56	<0.001
Scopulariopsis chartarum	45	59	0.67	<0.001
Stachybotrys chartarum	23	39	0.40	<0.001
Trichoderma viride	83	46	2.59	<0.001
Wallemia sebi	96	83	1.12	0.522
Group 1 Means	*66*	*55*	*1.43*	
Group 2				
Alternaria alternata	90	90	0.31	<0.001
Acremonium strictum	82	68	1.08	0.63
Aspergillus ustus	66	49	1.26	0.046
Cladosporium cladosporioides Type 1	85	99	0.26	<0.001
Cladosporium cladosporioides Type 2	82	76	0.72	0.01
Cladosporium herbarum	97	93	0.93	0.16
Epicoccum nigrum	83	96	1.89	0.002
Mucor group	88	94	0.25	<0.001
Penicillium chrysogenum Type 2	85	79	0.61	0.016
Rhizopus stolonifer	17	35	0.78	<0.001
Group 2 Means	*78*	*78*	*0.81*	

TABLE 4
Results of the Mold Specific Quantitative PCR Analysis of VB and SD Samples in 21 Homes with Median Rank

	SD ERMI	VB ERMI	SD Median Rank	VB Median Rank
1	−8.30	−8.46	1	1
2	−3.80	2.66	1	1
3	−1.66	0.34	1	1
4	−1.13	11.19	1	2
5	0.06	−3.54	1	1
6	0.59	3.35	1	1
7	1.05	−4.82	1	1
8	1.83	11.50	1	2
9	2.30	4.60	1	1
10	2.31	11.05	1	2
11	3.63	12.27	2	2
12	4.05	4.22	2	1
13	4.38	8.97	2	2
14	5.56	14.69	2	2
15	6.67	1.33	2	1
16	7.56	2.02	2	1
17	8.73	11.91	2	2
18	9.56	13.07	2	2
19	10.30	14.97	2	2
20	12.18	12.14	2	2
21	12.84	24.06	2	2
Medians	*3.63*	*8.97*		

on 36 common indicator species. Testing the AHHS samples for additional species indicated that these 46 were comparatively rare. Therefore, the need of incorporating more species into the ERMI was found to be unnecessary.

We recognized that sometimes it may not be practical to analyze all 36 ERMI species and that a less expensive screening of the home for mold burden, before a more thorough analysis is made, might be useful. The ARMI, using only 12 species, showed reasonable consistency with the ERMI in identifying homes with potentially problematic mold burdens. The ARMI does not, however, provide the depth of information about the species of molds present in a home as the ERMI but it may still be useful as a preliminary screening tool based on the relatively small sample of 21 recently sampled homes.

The analysis of only the 12 species of the ARMI scale was not sufficient to place a home into the same quartile as the analysis of the 36 species of the ERMI scale. Nevertheless, the reduced analysis was fairly accurate in median rank classification (ie, categorizing homes into either the lower or upper 50% of homes for mold burden). In some situations, this may be a reasonable first step to assessment. Those homes in the highest mold burden category will probably warrant the full ERMI analysis.

We also recognized that it is not always possible to obtain the SD sample which requires someone to come into a home and vacuum with a Mitest sampler. Therefore the possibility of substituting the VB sample dust was considered. There is much less control of a sample obtained from the VB, since it is based on the individual homeowner's habits. However, a VB is readily and inexpensively obtained from the average homeowner.

Based on comparison of the ERMI values obtained from the SD and VB samples collected from the same

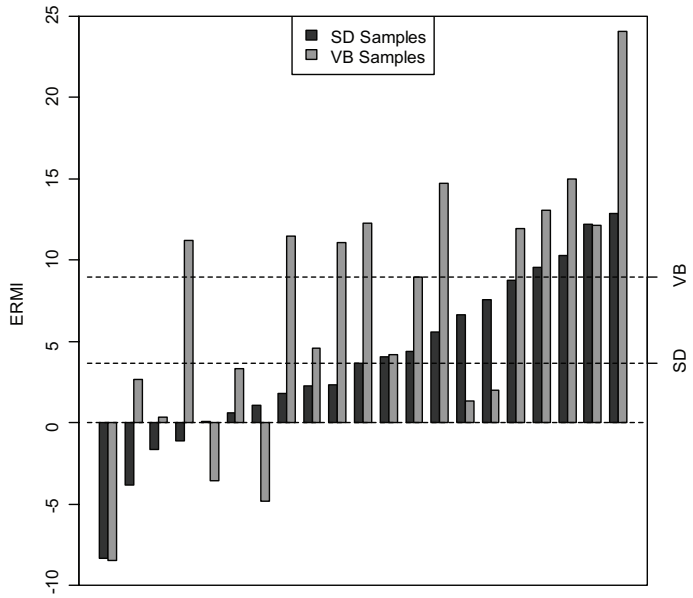

Fig. 3. Bar graph comparing the SD and VB Environmental Relative Moldiness Index (ERMI) values. Horizontal reference lines are median values. In 15 of 21 (71%) cases the VB samples place the ERMI value at the same median rank (Table 4).

homes, the VB sample was fairly reliable in placing a home into the category of the highest 50% or lowest 50% of homes on ERMI scale. For some screening purposes, this may be sufficient. Other studies have shown that vacuum cleaner dust is useful in describing the environmental conditions of homes.[8] Hyvärinen et al. measured various indicators of microbial contamination in vacuum cleaner dust to characterize the relationship between home conditions and asthma.[9]

The results of the Institute of Medicine survey of the literature surmised that mold exposure was linked to some asthma.[1] Recent epidemiological studies have continued to support this view.[10,11] Fisk et al. completed a meta-analysis of studies associating mold contamination with adverse health effects.[12] They concluded that building dampness and mold were associated with approximately a 30% to 50% increase in a variety of respiratory and asthma-related health outcomes. Other studies have shown that remediating the water-damage and mold in asthmatics' homes resulted in improvements in the asthmatics' health.[13,14] Therefore, assessing the mold burden in homes has become desirable. Although the full ERMI analysis using the SD sample provides a more precise estimate of the mold burden, application of the reduced ARMI scale and VB dust may be useful as screening tools.

TABLE 5
Results of the Mold Specific Quantitative PCR Analysis of 21 Test Homes for SD Samples Comparing the ERMI Values with ARMI Values on Median Rank

	ERMI	ARMI	ERMI Median rank	ARMI Median rank
1	−8.30	0.92	1	1
2	−3.80	−7.95	1	1
3	−1.66	2.13	1	1
4	−1.13	1.11	1	1
5	0.06	−0.31	1	1
6	0.59	1.56	1	1
7	1.05	1.82	1	1
8	1.83	3.04	1	2
9	2.30	3.88	1	2
10	2.31	1.66	1	1
11	3.63	3.69	2	2
12	4.05	1.88	2	1
13	4.38	2.79	2	2
14	5.56	2.98	2	2
15	6.67	4.12	2	2
16	7.56	2.17	2	2
17	8.73	2.12	2	1
18	9.56	3.14	2	2
19	10.30	3.76	2	2
20	12.18	11.78	2	2
21	12.84	7.95	2	2
Medians	*3.63*	*2.17*		

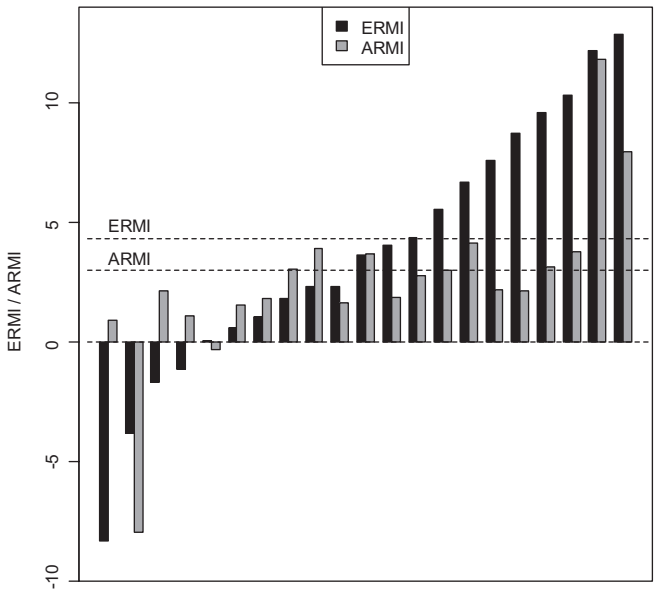

Fig. 4. Bar graph comparing Environmental Relative Moldiness Index (ERMI) and American Relative Moldiness Index (ARMI) values from the same home. Horizontal reference lines are median values. In 17 of 21 (81%) cases the ARMI value at the same median rank as the ERMI (Table 5).

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